RECOMBINANT DNA RESEARCH
Volume 18

Documents Relating to "NIH Guidelines for Research Involving Recombinant DNA Molecules" June 1993–December 1993

January 1995

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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January 1995

Prepared by the
Office of Recombinant
DNA Activities

NIH Publication No. 95–3904

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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PREFACE

This is the eighteenth in a series constituting a "public record" of activities in regard to the National Institutes of Health (NIH) Guidelines for recombinant DNA research.

The first seventeen volumes cover events from February 1975 through June 1993. This eighteenth volume covers events from June 1993 through December 1993.

Starting from Volume 14, human gene transfer/therapy protocols that have been approved by the NIH Recombinant DNA Advisory Committee, are collected. Documents of each human study include the clinical protocol, the Informed Consent document describing the study in a nontechnical language, both scientific and nontechnical abstracts, and curriculum vitae of the principal investigators.

Volumes 1 through 5 in this series and the Environmental Impact Statement may be purchased from the Superintendent of Documents, U.S. Government Printing Office (GPO), Washington, DC 20402 or GPO bookstores in selected cities throughout the United States. They may also be viewed in some 600 public libraries of the GPO depository system. The GPO stock numbers are: Volume 1, 017-004-00398-6; Volume 2, 017-040-00422-2; Volume 2 Supplement (Environmental Impact Statement), 017-040-00413-3; Volume 3, 017-040-00429-0; Volume 3 Appendices, 017-040-0043-3; Volume 4, 017-040-00443-5; Volume 4 Appendices 017-040-00422-7; and Volume 5, 017-040-00470-2. Volumes 6 through 16 are available from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, MD 20892, USA.

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HUMAN GENE TRANSFER PROTOCOL

9306-049 Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *A Molecular Genetic Intervention for AIDS - Effects of a Transdominant Negative Form of Rev.*

Date of RAC Approval: 6/7/1993
Date of NIH Approval: 9/3/1993

16.0 Scientific Abstract

The HIV-1 Rev protein is encoded by a fully spliced viral mRNA synthesized early in virus infection and required for productive HIV replication (23). Rev facilitates the cytoplasmic appearance of unspliced viral mRNAs and plays a role in the regulation of virus latency (24,25). Mutations in a well-conserved leucine rich domain of Rev give rise to a defective protein that acts as a transdominant inhibitor of HIV replication, which could provide a potential anti-HIV therapy. In this study, we will evaluate the efficacy of intracellular inhibition of HIV infection with a with mutant form of Rev, M10. We will introduce the rev M10 gene into peripheral blood $CD4^+$ T cells. $CD4^+$ cells will be genetically modified in patients using either (a.) a retroviral vector or (b.) a non-viral vector. In each case, a control vector identical to the Rev M10 expression vector but with a frameshift that inactivates gene expression will be used to transduce a parallel population of $CD4^+$ cells. These cells will be returned to the patient, and the survival of the cells in each group compared by limiting dilution PCR. In this way, we will determine whether Rev M10 can prolong the survival of $CD4^{\oplus}$ cells in AIDS patients, thus conferring protection against HIV infection.

17.0 Non-technical Abstract

Infection by the human immunodeficiency virus (HIV) leads to progressive loss of the body's immune defenses against infectious agents. Persistent HIV infection therefore leads to an acquired immunodeficiency syndrome (AIDS), for which there are currently no curative treatments. It has recently become clear that the introduction of specific genes into T cells can provide protective effects against HIV growth in the laboratory. One of these protective genes is an altered inactive form of an essential viral gene, called Rev. Expression of this altered form of Rev, called Rev M10, inhibits normal Rev function and blocks productive HIV replication. In this study, we propose to determine whether Rev M10 can improve the survival of T cells in humans. If this approach proves successful, it could provide the basis to develop Rev M10 as a molecular genetic intervention for the treatment of AIDS.

"A Molecular Genetic Intervention for AIDS--Effects of a Transdominant Negative Form of Rev"

Protocol

HUMAN GENE THERAPY PROTOCOL

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Submission Date: April 12, 1993

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1.0 Introduction

1.1 Background

Infection by the human immunodeficiency virus (HIV) leads to progressive depletion of $CD4^+$ T cells which causes an acquired immunodeficiency syndrome (AIDS). Over the past decade, AIDS infection has achieved epidemic proportions. Despite considerable progress in understanding and diagnosing this disease, it has remained refractory to treatment. There are currently no effective means to vaccinate susceptible individuals or to treat infected patients. Infection by the human immunodeficiency virus is typically characterized by an asymptomic, or latent, phase of the disease. During this time, patients may not exhibit signs of immunodeficiency but nonetheless synthesize virus which progressively depletes $CD4^+$ T cells and is infectious. It is currently estimated that there are over 1 million seropositive cases with latent HIV infection in the United States today. These individuals, for whom there are no known effective treatments, are likely to progress and succumb to this disease.

For these individuals, a major therapeutic goal is to prolong the latent phase of HIV infection. A major interest of our laboratory has been to define the molecular basis of HIV gene activation in T lymphocytes. We have previously shown that activation of HIV gene expression is controlled by a cellular transcription factor, NF-κB (1). This transcription factor is inactive in resting T cells but is stimulated following cell activation and induces viral transcription. In addition to NF-

κB, there are essential viral genes which also appear to regulate the transition from latent to active infection. An important viral regulatory protein in this process is Rev, an 18 kd nuclear protein which controls export of viral RNA from the nucleus to the cytoplasm of infected cells. In contrast to NF-κB, the Rev protein is unique to the virus and unlikely to regulate essential cellular functions. It therefore becomes a preferred target for anti-viral gene therapy.

Viral replication is critically dependent on the interaction of viral gene products with host cell factors. Because viruses are intimately associated with their host cells, it has been difficult to selectively interfere with replication *in vivo*. Successful anti-viral approaches have selectively targeted viral gene products. For example, the treatment of herpes simplex virus (HSV) infection has taken advantage of the ability of a viral gene, thymidine kinase, to modify a drug which is toxic to the host cell. This approach led to the development of guanosine analogues, including acyclovir and ganciclovir (2-4), which are converted to DNA chain terminators only in HSV infected cells.

In HIV infection, traditional pharmaceutic targeting has thus far provided limited benefits. Although AZT has relative selectivity for viral reverse transcriptase, its toxic effect on host cell function and its low therapeutic index has provided limited protection against the progression of AIDS.

Although drug discovery has led to the identification of additional anti-viral drugs, the high rates of virus mutation has lead to the generation of resistant viral mutants. More

recently, as molecular biologic studies have advanced, it has become possible to use recombinant genes to interfere with HIV gene expression. Several approaches have been used to exploit gene transfer to inhibit viral replication. These include anti-sense RNA (5-7), catalytic RNA, or ribozymes (8-11), and RNA analogs or decoys (12). The gene transfer approach to targeting of viral nucleic acid is complicated by problems of delivering the anti-viral gene to the appropriate compartment of the cell, and by the ability to obtain catalytic activity *in vivo*. These approaches, although promising, have thus far been difficult to achieve. In contrast, viral proteins are well-known and contain domains which can be characterized with respect to structure and function. These viral proteins can therefore serve as targets for inhibition by recombinant gene products.

The concept of dominant negative inhibition was initially described in yeast genetic systems (13). It was subsequently demonstrated that anti-viral effects could be conferred on cells susceptible to infection by herpesvirus. Using the herpesvirus VP16 transactivator, mutant proteins lacking the transactivation domain of this protein were generated which could interfere with viral replication (14). The approach to dominant negative inhibition is an attractive one for the treatment of AIDS. For HIV, several potential transdominant proteins have been defined which have been successful in transient transfection systems to inhibit viral replication. Among these are the rev transdominant protein (15) and viral group-specific antigens (GAG). Recent success in protecting cells from HIV infection using TAR analogs

has provided additional evidence that it is possible to render cells resistant to HIV infection (12) through recombinant gene products, and has provided further incentive to pursue additional molecular genetic interventions proteins which can achieve this effect.

2.0 Objectives

Replication of human retroviruses is dependent upon virally encoded transactivator proteins. These proteins often interact with host cell factors and specifically modulate host cell processes. One approach to the treatment of viral infections is to inhibit the function of essential viral genes by expression of transdominant forms of viral transactivators which inactivate them. The rev gene of HIV represents one such essential viral regulatory protein. Specific mutations with the coding sequence of Rev render the virus replication defective. We have previously shown that a dominant negative form of Rev, M10, inhibits productive viral replication but does not appear to interfere with normal cellular functions.

In this proposal, the effects of stable expression of a Rev transdominant protein will be assessed in peripheral blood T lymphocytes in patients. Expression of this protein conferred substantial resistance to HIV infection in stably transduced T cells in the laboratory, without affecting induction of HIV transcription mediated by either the κB regulatory element or Tat within CEM M10 subclones. In EL-4 and Jurkat leukemia lines, constitutive expression of M10 did not affect the induction of IL-2 secretion by mitogens. We have optimized the delivery of this gene to T lymphoid cells, examined its resistance to infection by primary viral isolates, and assessed its effect on T cell function. These studies suggest that Rev M10 may provide a therapeutic effect in AIDS patients. The emphasis of this proposal is to direct expression to peripheral blood lymphocytes

to evaluate whether this treatment could be therapeutic. At the same time, these studies may also determine whether it is appropriate to express this gene, alone or in combination with others in hematopoietic stem cells in the future.

The aim of this proposal is to determine whether expression of a specific recombinant gene can prolong the survival of peripheral blood lymphocytes in AIDS patients. This recombinant gene is derived from the rev gene of the human immunodeficiency virus (HIV). A mutant form of rev, Rev M10, inhibits productive virus replication in cells susceptible to HIV infection. We will introduce a retroviral vector encoding Rev M10 and a control vector containing a frameshift mutation leading to an untranslated mRNA (ΔRev M10) into peripheral blood lymphocytes with retroviral vectors to determine:

1) whether expression of Rev M10 in peripheral blood lymphocytes improves cell survival in comparison to control cells transduced with ΔRev M10.

2) the effect of this treatment upon viral replication and immune function *in vivo*.

3.0 Selection of Patients

Patients will be carefully selected based on consideration of their past medical history and present status. The referring physician, the attending physician, and infectious disease or the relevant clinical department, the patient, and family members will make a joint decision regarding the inclusion of the patient in this trial. The following criteria will be used to select appropriate patients for study:

3.1 Criteria for patient eligibility

3.1.1 Patient must be HIV seropositive.

3.1.2 Patient must be greater than 18 years of age.

3.1.3 The patients must be treated with AZT.

3.1.4 Patient must have a CD4 count greater than 250.

3.1.5 The patient must be able to provide informed consent.

3.2 Criteria for patient ineligibility

3.2.1 Patients with active autoimmune disease.

3.2.2 Patients with an acute infectious illness within one month of initiation of treatment.

3.2.3 Patients with active hepatitis, chronic or acute.

3.2.4 Patients with diabetes mellitis who are not controlled by medical treatment.

3.2.5 Psychiatric illness which makes compliance to the clinical protocol unmanageable.

3.2.6 Patients on high dose glucocorticosteroids.

3.2.7 Patients who undergo an alternative mode of anti-HIV treatment will not be accepted within 4 weeks of the last treatment.

4.0 Clinical Evaluation Prior to Treatment

As described above, patients will be selected based on their past medical history and clinical course. Patients must have a confirmed HIV infection documented by Western blot analysis. The patients must meet the standard inclusion and exclusion criteria described above. In addition, the chosen patients must show no signs of active systemic infection or major cardiovascular or respiratory disease. The following laboratory values will be used as guidelines as enrollment:

1. The bilirubin should be less than 2 mg/dl.
2. The platelet count should be greater than $100,000/mm^3$.
3. The white blood cell count should exceed $3,000/mm^3$.
4. The creatinine should be less than 2.0 mg/dl.

5.0 Stratification and Randomization

Not relevant.

6.0 Nature of Procedures or Therapeutic Agents

6.1 Study Design.

This study is designed to determine whether the introduction of a transdominant form of Rev will prolong the survival of T lymphocytes *in vivo* which contain this gene. If resistance can be conferred upon these cells to HIV infection *in vivo*, then it will warrant further studies to optimize this molecular intervention for therapeutic purposes in AIDS patients. Briefly, the Rev M10 gene will be introduced into $CD4^+$ cells after depletion of mononuclear cells and B cells using soybean agglutinin and removal of $CD8^+$ cells with antibody-coated plates (see Appendix IV). These $CD4^+$ cells are ~90% homogenous and will be stimulated *in vitro*. Two methods of stimulation will be used, each in a separate patient. In the first instance, cells will be stimulated with anti-CD3 and IL-2 as by standard methods (Appendix IV). In the second case, cells will be stimulated with antibodies to CD3 and CD28 coated to tissue culture plates. These antibodies stimulate different signal transduction pathways to T cells, and have different growth stimulation properties. For example, CD3 and CD28 stimulation provide preferential proliferation of $CD4^+$ cells while stimulation with CD3 and IL-2 stimulates both CD4 and CD8 populations simultaneously.

In each case, a control gene will be introduced into a separate aliquot of cells which contain a frameshift which abrogates Rev M10 expression (ΔRev M10). We will first analyze the effects of Rev M10 or ΔRev M10 on T cell survival after introduction into T cells by retroviral vectors. The structure

of the retroviral vector is described in the "Response to the Points to Consider." Briefly, it utilizes the MoMuLv enhancer to stimulate expression of the Rev M10 gene. In the control, a population of cells derived from the same isolation procedure will be transduced within a nearly identical retroviral vector which contains a frameshift at the initiation code of Rev M10 and does not produce a functional gene product. The half-life of cells which express Rev M10 will be compared to those which lack the open reading frame. These two populations will be distinguished using a limited dilution PCR of cells derived from the peripheral blood with primers specific to each vector. In this way, the numbers of cells which persist as a function of time will be determined, and the half-life of cells transduced with each vector will be assessed. In addition, differences in survival of cells stimulated by αCD3 and IL-2, vs. αCD3 and αCD28 will be compared.

In a second group of patients, the Rev M10 gene will be introduced with a non-viral vector, using biolistic injection with linearized plasmids in T cells. In this case, a plasmid containing the Rous sarcoma virus enhancer linked to the TAR region of HIV driving expression of M10 will be used, since we have found it to show greater efficacy in electroporation experiments (Section 13, Preliminary Data).

Patients will be admitted to the Clinical Research Center at The University of Michigan Medical Center after the relevant eligibility criteria have been met. The pre-treatment evaluation will be performed as described in Section 9. Patients undergoing

the gene transfer protocol will have serum samples removed for analysis as described in Section 9. PBL and serum will be stored frozen for further analysis and PCR.

After treatment, the patients will be observed in the Clinical Research Center for 24 hours, and blood collections performed as described in Section 9. If there are no complications, the patient will be discharged after 24 hours. Should any abnormalities appear, the patient would be kept for further observation.

6.2 Confirmation of Gene Transfer and Expression

An aliquot will be processed to obtain DNA for PCR analysis. Limiting dilution PCR will be performed by standard methods. Reverse transcription PCR analysis will also be performed to confirm expression of the Rev M10 and ΔRev M10 transcripts.

6.3 Analysis of Immune Parameters

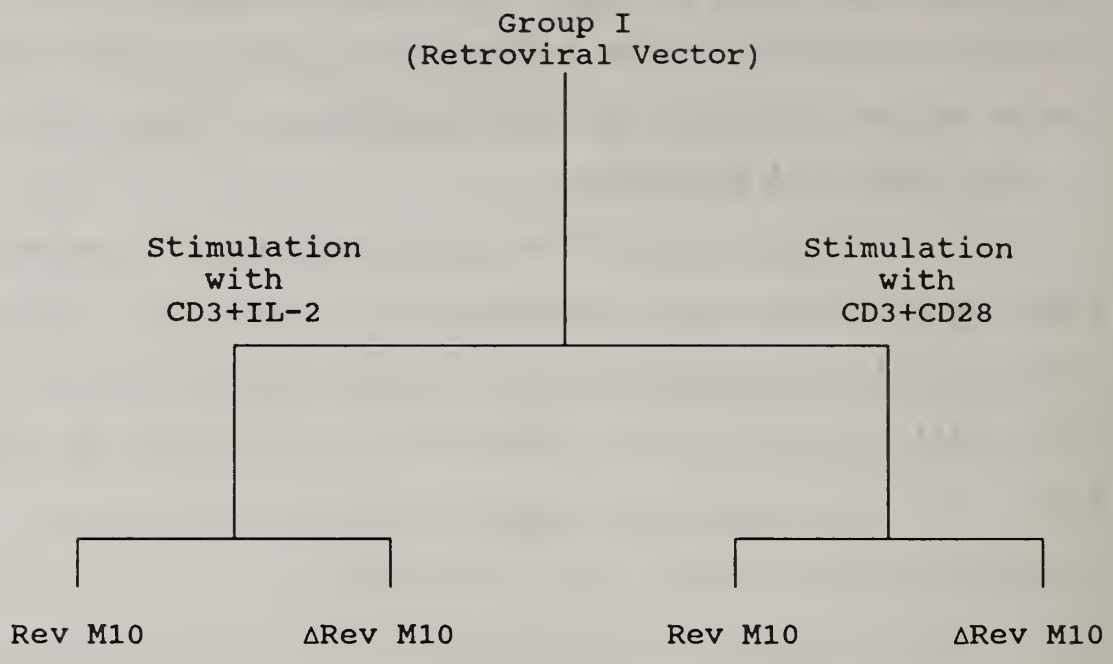
We will monitor immune parameters in these patients to determine whether the treatment exerts any therapeutic effects on the immune system. We will also monitor patients by an immune response to Rev M10 itself. To perform this analysis, a blood sample will be obtained prior to treatment to derive lymphocytes which will be immortalized using the Epstein-Barr virus. An aliquot of these cells will be further infected with an amphotropic Rev M10 retroviral vector (16), and selected for G418 resistance. Expression of Rev M10 will be confirmed by RNA analysis. These cells will subsequently be used in the laboratory as target cells for the cytolytic T cell assay. At no time will these cells be brought into the same building where the

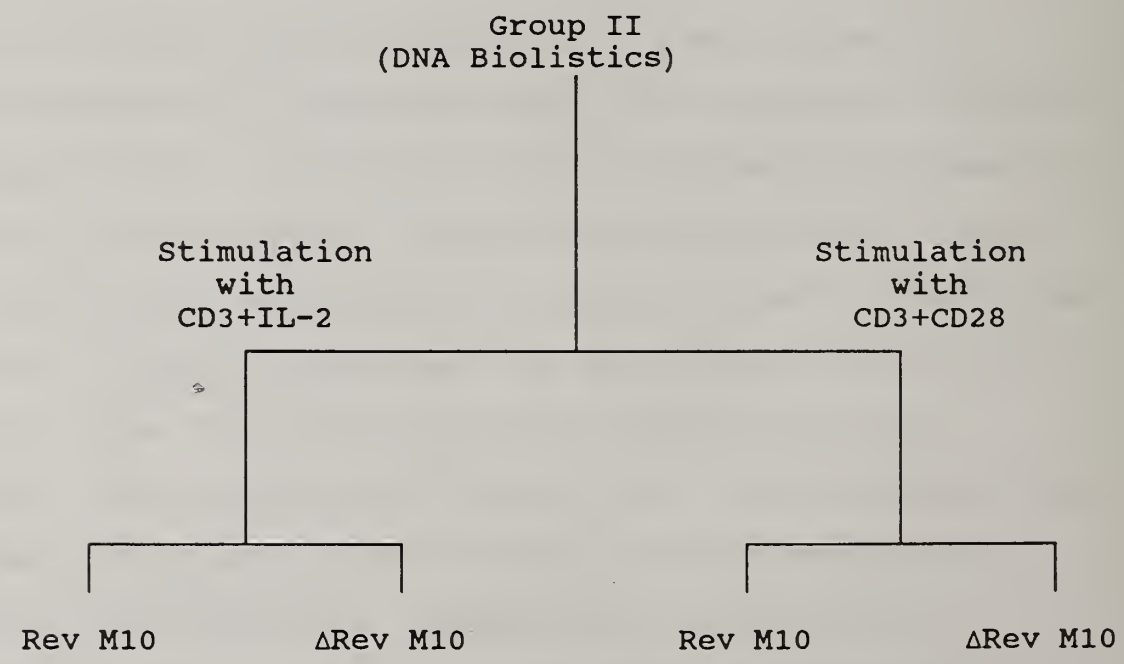
patient is being treated. These cells and laboratory experiments will be performed in MSRB II, Room 3560, whereas the patient's clinical treatment will be taking place in the Clinical Research Center of The University of Michigan Medical Center Hospital.

6.4 Repeated Treatment

If no adverse side effects of the treatment are observed, repeated infusions will be considered at two-month intervals. Doses identical to the initial treatment regimen will be repeated with similar protocols and observation as described in Section 6.1.

7.0 Schema/Duration





8.0 Dose of Therapeutic Modifications

Patients can receive medications to control any side effects of treatment. Such agents would include acetaminophen (650 mg. q. 4 hrs.), indomethacin (50-75 mg. q. 6 hrs.), or antihistamines as required. Glucocorticoids will not be used in these patients; or if such medication is required, the patient will be removed from the protocol.

9.0 Study Parameters (revised)

Parameters

		During Treatment							
		Day				Week			
	Pre-Study	1	7	14	28	8	12	16	20
Physical exam	X	X*	X	X	X	X	X	X	X
History	X								
Performance Status	X						X	X	X
Chemistry Survey[1]	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X
CBC, Diff, Platelet	X	X	X	X	X	X	X	X	X
PT, PTT	X	X		X	X		X	X	X
EKG	X								
CXR	X								
U/A and Culture	X								
Hb_sAg, HIV	X								
Assess for adverse events status	X	X	X	X	X	X	X	X	X
PCR on PBL and serum to detect recombinant genes	X	X		X	X	X	X	X	X
Assay for Rev M10 cytotoxic T cells	X				X		X	X	X
Assay for Rev M10 antibody	X			X	X		X		
Pregnancy test (if relevant)	X								
CD4 counts	X				X	X	X	X	X
Viral load (RNA analysis)	X				X	X	X	X	X
ANA, ESR, CH_{50}									

* An additional sample will be taken several minutes after injection to examine for the presence of DNA in circulatory PBL.

[1] Routine laboratory tests include glucose, electrolytes, BUN, creatinine

9.1 Pre-treatment (see Table)

The following studies will be performed as summarized in Table 1.

a. Complete physical examination noting in detail any infections that might exist.

b. Chemistry survey including electrolytes, liver function tests, calcium, magnesium, creatinine, BUN, CPK, pancreatic enzymes.

c. CBC, differential count, PT, PTT, platelet count.

d. Urine analysis and culture.

e. Hepatitis screen.

f. HIV titer.

g. Chest x-ray.

h. Electrocardiogram.

i. 45 ml of clotted blood for serum storage and 45 ml of anti-coagulated blood for mononuclear cell cryopreservation.

9.2 Treatment

Patients will be analyzed with routine blood and chemistry analysis. A chest x-ray will be initially subsequently if needed. During the gene transfer protocol, patients will be monitored closely in the Clinical Research Center. Vital signs will be measured every 15 minutes prior to, during, and after the infusion for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80 mm/Hg or the oxygen saturation drops below 90%, the infusion will be terminated immediately.

9.3 Post-treatment

Patient's status will be evaluated by physical examination, laboratory studies, and appropriate imaging scans prior to treatment, and monthly after the first month or as needed to

evaluate response to treatment. Serum will be analyzed by PCR for the presence of plasmid, and antibody to Rev M10 will be evaluated. PBL's will be isolated and analyzed for their ability to lyse Rev M10 modified autologous EBV-transformed lines. If ANA becomes positive or other signs of autoimmunity appear, evidence of more specific autoantibodies, e.g., anti-Rho, Smith, etc., will be tested and rheumatology consultation will be sought.

9.4 Potential Side Effects and Reporting of Adverse Reactions

Adverse Drug Reactions will be reported to the Drug Information Service, University of Michigan Medical Center, phone 313-936-8200 or 313-936-8251 (available 24 hours), and include all life-threatening events (Grade 4) which may be due to drug administration, all fatal events, or the first occurrence of any previously unknown clinical event (regardless of Grade). A written report is to follow within 10 working days to:

Investigational Drug Branch
P.O. Box 30012
Bethesda, Maryland 20824
Phone: 301-496-7957

Data will be submitted to the IRB monthly or upon request. All adverse reactions will be reported to the IRB, even if there is only a suspicion of a drug effect. All side effects will be graded using the standard toxicity sheet described in Section 14, Common Toxicity Criteria.

9.4.1 Potential Risks of Gene Transfer In Vivo

Insertional mutagenesis. The possibility of causing malignancy in cells secondary to the random insertion of the DNA in the genome exists, though this risk is considered low (17-19). There is a remote chance that the vector could replicate in the host when FDA recommended procedures are not followed, and it is essential to screen all retroviral vector lots for replication-competent helper virus using the S^+L^- assay, testing ~1/10 of each lot. When these guidelines have been followed in the past, there have been no complications detected in immunodeficient individuals. Two additional features of the study design provides additional safety features for this trial. All patients will be maintained on AZT, which exerts an anti-viral effect on murine retroviruses. Therefore, replication-competent viruses would be inhibited by this agent. Finally, the use of a less extended packaging sequence without gag should minimize the risk of recombination and the generation of replication-competent virus. With DNA microprojectile injection, the risk of an adverse event is even lower, since it cannot generate an infectious agent. In either case, blood samples will be obtained after treatment of each. PCR and S^+L^- assays will be performed to monitor for this unlikely event. In the case of biolistics, we would expect the risks to be similar or less than retroviral vectors, and related to the potential for insertional mutagenesis.

The use of aminoglycoside antibiotics. The neomycin resistance gene, which encodes neomycin phosphotransferase (NPT),

phosphorylates the 3′ hydroxyl group of the aminohexose I of neomycin and its analogues, inactivating the antibiotic. While amikacin may be inactivated by this enzyme, gentamicin and tobramycin do not contain an hydroxyl at the 3′ position and are not inactivated. Therefore, introduction of the NeoR gene would not exclude the use of aminoglycosides or any other conventional antibiotic that may be needed in the clinical management of these patients.

Although risks to the patient exist in this study, they would appear to be minimal, and the nature of the vectors and concurrent medications should minimize the risks to any individual patient. Because there are no curative treatments for these patients, these risks are thought to be justified, considering the potential therapeutic benefit, either to them or to future patients.

10.0 Off-Study Criteria

Patients will be removed from the study should any grade 3 or 4 toxicity develop which is not easily corrected. The toxicity sheet is included in Section 14.0, Common Toxicity Criteria). If two treatment-related deaths should occur, this protocol will be terminated.

11.0 Evaluation of Results

11.1 Criteria for Response

There are three primary clinical endpoints in this study. First, we will determine whether $CD4^+$ T cells which express the Rev M10 gene have enhanced survival in patients with chronic HIV infection. The presence of these genetically modified cells will be quantitated using a limiting dilution PCR technique in which cells from the patient are diluted with a fixed number of untransfected control cells and the frequency of positive cells is determined as a function of time. Since a parallel population of cells will be transduced with a control vector in which the Rev M10 gene product is not synthesized, the survival of the two populations can be compared.

A second endpoint is to characterize the immune response of the host against the Rev M10 gene product. We will determine whether antibodies are generated to Rev M10 by Western blot and immunoprecipitation analyses. In addition, we will determine whether cytolytic T cells are induced in patients which recognize cells containing Rev M10. Although Rev M10 is a nuclear protein and no immune cells have been detected previously in animal models, it is not known whether the expression of this gene might affect the immune system differently in man. Such a response would interfere with the constitutive expression of the dominant negative protective gene. Should this occur, however, it would be important to know it, since it might provide a strategy to enhance immune recognition of cells latently infected with HIV.

Finally, although we do not expect that the relatively small number of cells in this study will confer protective effects on immunologic function, we will nonetheless examine CD4 counts and viral load using a commercially available assay to quantitate serum viral RNA levels (Chiron).

11.2 Confirmation of Recombinant Gene Expression

Several independent techniques will be used to evaluate the presence and expression of the recombinant gene *in vivo*. We have used several antibodies to Rev to detect the recombinant gene product *in vivo* by immunoprecipitation (16). The presence of retroviral or plasmid DNA will be confirmed by PCR of DNA from peripheral blood lymphocytes or in selected lymph node specimen tissue, if available. RNA will also be isolated and examined for the presence of Rev M10 or ΔRev M10 mRNA by reverse transcription PCR or S1 nuclease analysis.

11.3 Analysis of Immune Response

Cytolytic T cell activity will be evaluated by incubation of peripheral blood lymphocytes with ^{51}Cr labeled Rev M10 transduced EBV lines from the patient. The presence of antibody will be evaluated by FACS analysis of a matched pair of Rev $M10^{+}$ or ΔRev M10 cell lines. In some instances, lymphocytes will be isolated, expanded in tissue culture, and analyzed for cytolytic function.

11.4 Toxicity

Toxic side effects of treatment will be analyzed and classified by the common toxicity criteria (Section 13).

12.0 References

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13.0 Preliminary Data

I. T Cell Dependence and Specificity

In previous studies, we and others have documented the effectiveness of the Rev M10 gene as a dominant negative inhibitor of productive HIV replication (15,16,20). We have also shown that expression of Rev M10 in chronically infected T leukemia lines leads to protection against HIV infection without altering immune function (16). Although protection can be overcome with high titers of virus, several orders of magnitude of protection can be conferred, similar to antiviral agents which are already in clinical practice. This experiment has now been performed with 10 independently derived Rev M10 cell lines using retroviral vectors (16, see also below), and by electroporation with Rev M10 linked to different enhancer sequences (see below).

To determine whether expression of Rev M10 could provide resistance to HIV infection in human peripheral blood lymphocytes, we utilized a retroviral vector containing a G418 resistance gene. When cells were transduced with this retroviral vector and selected for 8 days or more prior to HIV infection *in vitro*, the cells which expressed Rev M10 showed a marked degree of protection from retroviral infection compared to the identical vector which contained a mutation that did not allow the protein product of Rev M10 to be made (ΔRev M10). These data document that expression of Rev M10 in human peripheral blood lymphocytes confers protection against HIV infection *in vitro*. This protection is found both with a cloned viral isolate, as well as with a freshly passaged clinical isolate *in vitro*. A

representative experiment is shown in Fig. 1. It is important to note that a non-specific inhibitory effect is seen when cells are grown in the presence of G-418. This effect has been described by many laboratories. In addition to this effect, Rev M10 shows the ability to inhibit productive viral replication compared to cells containing the frameshift Rev M10 negative control.

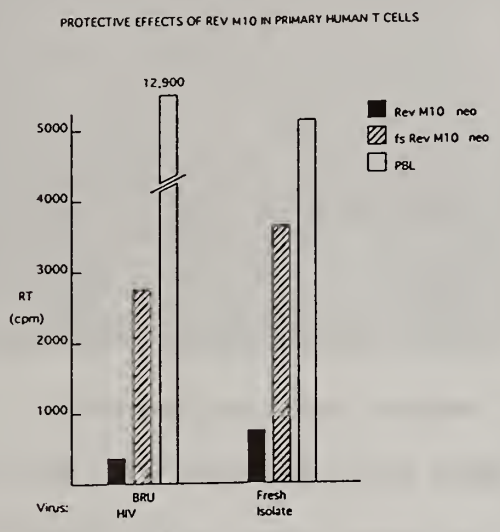


Figure 1. Freshly isolated PBMC's were stimulated for 48 hours at 37°C with 5μg/ml PHA. On day 3 and 5, cells were infected for 8 hrs with ψcrip supernatants, (plus 5μg/ml polybrene) containing the PLJ-Rev M10 neo or frameshift PLJ-fsRev M10(neo) retrovirus. Cells were subsequently selected with 300μg/ml G418 for a further 8 days. On day 8, 5 x 10^6 cells in 0.5ml of medium were incubated with approximately 5 x 10^4 $TCID_{50}$ of HIV ^{bru} or a freshly passaged clinical isolate for 2hrs at 37^C. Cells were washed and resuspended at 1 x 10^6 /ml. Day 7 post HIV infection, duplicate culture supernatants (10μl) were assayed for reverse transcriptase activity.

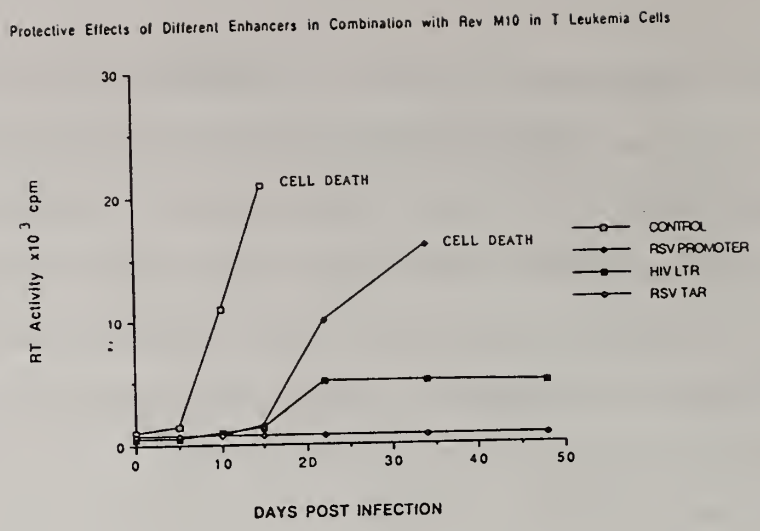


Figure 2. Control CEM cells and CEM cells stably expressing RevM10 from various enhancers (RSV, HIV LTR or RSV-tar), were infected with HIV^{Bru} (1:500 ratio $TCID_{50}$ HIV:CEM cells), for 2 hrs. Cells were then washed and resuspended in fresh media. Culture supernatants were then assayed for RT activity at various times post infection. Cultures were split every 3 to 5 days dependent on growth rate. The plasmid proposed for use in the biolistics protocol is RSV-tar, whose structure and sequence is described in Appendix V.

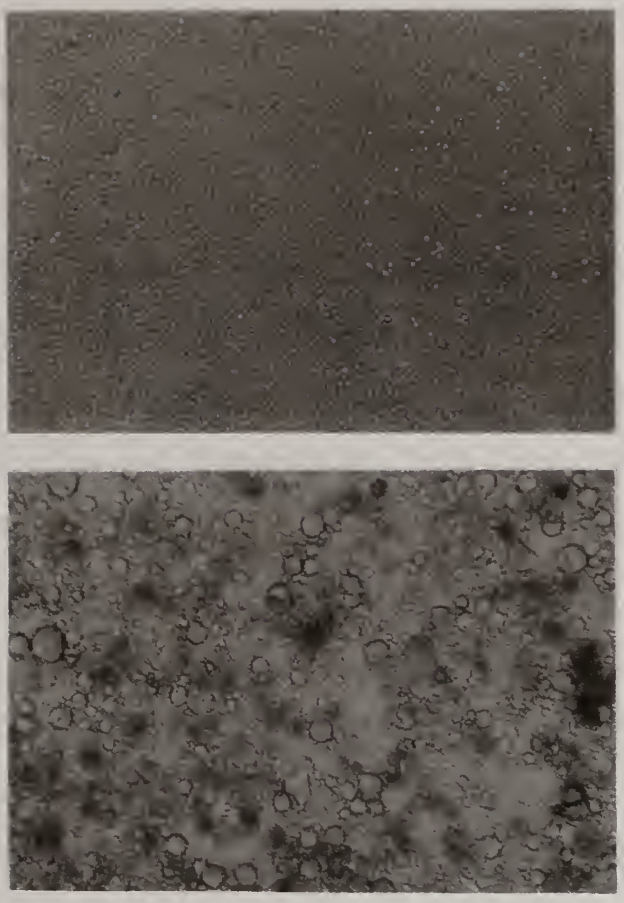


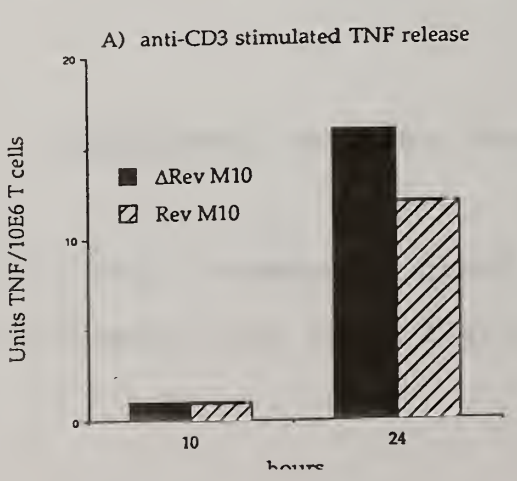
Figure 3. Protective effect of RevM10 expressed in T Leukemia cells to HIV infection.

Control CEM cells and CEM cells stably expressing RevM10 (RSV/TAR construct) were infected with $HIV^{Bru.}$ Cells were then washed and monitored for cytopathic effects. Control cells showed syncytia formation 5 days post infection. RevM10 expressing cells showed no CPE for more than 50 days of cell culture.

II. Expression of Rev M10 does not alter immunologic function in nontransformed cells.

The expression of Rev M10 in human T leukemia lines had no effect on several different T leukemia lines with respect to NF-

κB activation and the function of the HIV tat-I gene. In two cell lines, EL-4 and Jurkat, the expression of Rev M10 did not alter the ability of these cells to produce IL-2 following mitogen stimulation (16). In addition, no changes have been detected in any of these cell lines with respect to cell proliferation or cell surface antigen expression, including CD4 and CD3 (data not shown). We have also examined the effects of constitutive Rev M10 expression in primary T lymphocytes of murine or human origin. Genetically modified human peripheral blood T cells resistant to HIV infection were assayed for immunologic function. Rev M10 and ΔRev M10 transduced-selected peripheral blood T cells were assayed for tumor necrosis factor α secretion (TNFα) following stimulation with immobilized anti-CD3. No differences in the level or kinetics of TNF cytokine secretion were observed between the two populations (Fig. 4, panel a). The cytolytic activity of these same populations was assayed by anti-CD3 directed killing of P815 tumor targets. In these experiments, both Rev M10 and ΔRev M10 populations of T cells exhibited similar levels of cytotoxicity (Fig.4, panel b). Rev M10 transduced cells also showed the ability to secrete interferon γ (IFNγ) (data not shown).



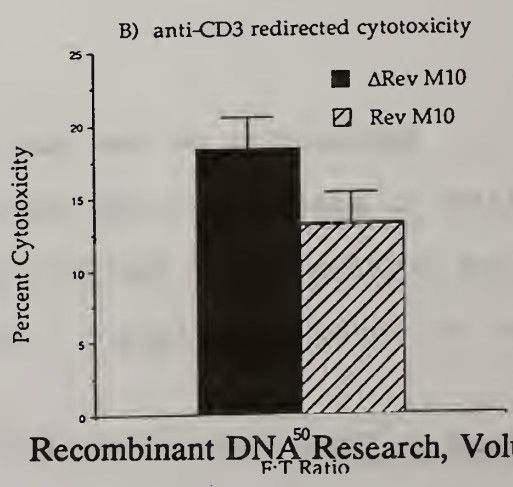


Figure 4. Analysis of immunologic function of primary T cells after transduction with Rev M10 or ΔRev M10 vectors. T cells were stimulated with immobilized anti-CD3 and supernatants harvested after 10 and 24 hours of culture. (A) Supernatants were analyzed for TNFα activity using the L929 bioassay. (B) Cytolytic function of transduced T cells were evaluated using anti-CD3 crosslinking of T cells to Fc receptor positive ^{51}Cr-labeled P815 tumor targets.

III. Expression of recombinant genes in T cells following biolistic injection

Although retroviral vectors are well-established as a means to introduce genes into T cells, successful transduction requires the activation of T cells which may accelerate their subsequent cell death. Because of this problem, we have begun to develop techniques to introduce DNA into primary human peripheral blood lymphocytes using electroporation. To accomplish this, unstimulated or activated cells were transduced with biolistic injection under conditions optimized for gene expression. The details of this protocol are included in Appendix IV, Section 8. When these experiments are performed, recombinant gene expression can be detected in 1-10% of human peripheral blood $CD4^+$ T lymphocytes. From this population, 0.1% of cells can be cloned by limiting dilution which show G418 resistance with the RSV-tar plasmid proposed for use in these studies. Experiments are on-going to determine whether these cells are resistant to HIV

infection. These results will be forwarded as soon as they are available.

IV. Activation of human peripheral blood lymphocytes in the presence of antiviral agents and lack of effect on retroviral transduction.

In order to transduce human peripheral blood lymphocytes with retroviral vectors and avoid activation of latent HIV, we have utilized conditions which are selectively cytotoxic for HIV. This strategy employs the use of non-nucleoside reverse transcriptase inhibitors which eliminate HIV *in vitro* but do not affect murine retroviral gene reverse transcription. These agents include B-HAP-E and CD4 pseudomonas exotoxin (Upjohn, Inc., Kalamazoo, MI) or niverapine (Boehringer Mannheim, Inc.). As previously published (21), incubation of these cells under these conditions prevents the activation of HIV. At the same time, no effects are seen on transduction using a retroviral vector encoding β-galactosidase (BAG) in the presence of these agents. Thus, these conditions will be used in the transfection protocol to introduce the recombinant genes into seropositive patients with HIV infection without activating latent virus.

Conc. Nevirapine nM	β-gal No. of Stained Cells
0	206
40	167
100	236
200	211
400	196

Table 1.
Effect of Nevirapine on Amphotrophic Retrovirus Integration

Amphotrophic pLJ-βgal supernatants (200 μl) produced from γCRIP cells were innoculated onto 293 cells growing in 8 well plates. The wells contained 2 mls of medium with the indicated concentrations of Nevirapine. After 3 days of incubation, cells were fixed and stained for β-gal activity by X-gal staining and the number of positive cells per 6 cm^2 well counted. Control transduced cells showed no X-gal staining. In addition, no toxicity of the drug was evident.

Days Post HIV Infection	RT Activity cpm + Nevirapine	Control
5	271	745
7	310	1950
10	165	5147
14	235	1861

Table 2. Effect of Nevirapine on HIV replication in PBL

Freshly isolated PBMC's were stimulated with PHA (5 μg/ml) for 48 hours, washed twice in fresh medium, and $5x10^6$ cells resuspended in 0.5 ml fresh medium. HIV was mixed with the cells ($5x10^4$ $TCID_{50}$) and incubated at 37°C for 2 hours. Cells were then washed once and resuspended in 10 ml fresh medium with or without 40 nM Nevirapine. At the indicated times, supernatants (10 μl) were assayed for RT activity. The original concentration (~ 40 nM) of Nevirapine was maintained throughout the experiment. Background cpm from uninfected cells alone, is approximately 200, with SD 50.

V. Animal models of HIV infection.

There is currently no suitable animal model for HIV infection which can be used to evaluate the efficacy of this treatment. Although there are non-primate model systems in which viremia can be achieved, the nature and virulence of these diseases differs significantly from those seen in man. It is therefore felt that the most suitable context in which to determine the efficacy of Rev M10 function is in humans. If successful, it would also allow for more rapid optimization for therapeutic efficacy. To adapt this study to an *in vivo* setting, we have adopted the model of HIV infection of human peripheral blood lymphocytes reconstituted in a SCID-HU mouse as described by Mosier et al. (22). In this system, we have transduced human peripheral blood lymphocytes with Rev M10 or ΔRev M10 retroviral vectors, selected for 8-15 days *in vitro* with G418. Resistance to HIV infection *in vitro* was confirmed, followed by adoptive

to HIV infection *in vitro* was confirmed, followed by adoptive transfer of these cells into SCID-HU mouse recipients followed by infection with virus *in vivo*. These studies are ongoing, and the results will be presented when available.

Finally, we have also attempted to model the experimental design in this murine system. A population of $CD4^+$ cells is transduced with the vector proposed for use in this study. When these cells are transduced in two populations and inoculated into the SCID-HU mice, their survival can be monitored *in vivo* by limiting dilution analysis. These studies are currently in progress, and their status will be summarized at the time of the RAC meeting if these results are available. Although we have utilized this animal model, it is clear that it is not a suitable model for HIV infection and its many manifestations in man. The definitive study can only be performed in humans.

University of Michigan Medical School
Department of Internal Medicine
and the Clinical Research Center

C O N S E N T F O R M

Informed Consent

(To be read by the Patient and explained
to the Patient by his or her Physician).

PROTOCOL: A Molecular Genetic Intervention for AIDS --
Effects of a Transdominant Negative Form of Rev

PRINCIPAL INVESTIGATOR: Gary J. Nabel, M.D., Ph.D.

ASSOCIATE INVESTIGATORS: Dennis Schaberg, M.D.

PROTOCOL NUMBER: _____ DATE: _____

PATIENT NAME: _____ HOSPITAL NO.: _____

INTRODUCTION

We invite you to participate in a research study at the University of Michigan Medical Center. Several general principles apply to all who take part in any experimental studies:

(1) Your participation in this study is voluntary
(2) You may not personally benefit from this study, but knowledge may be gained from it that will benefit others
(3) You may withdraw from the study at any time for any reason without jeopardizing your further care.

The nature of the study, the risks, inconveniences, discomforts, and other information are discussed in the following sections. Please do not hesitate to discuss any questions you have about this study with the physicians who explain it to you.

DESCRIPTION OF TREATMENT OR PROCEDURE TO BE UNDERTAKEN

As you are aware, you have an infection caused by the human deficiency virus (HIV). Infection by this virus leads to an acquired immunodeficiency syndrome, otherwise known as AIDS, which leads to weakening of your body's defense against infections, the immune system. At the present time, this disorder which cannot be cured by medicines. In this study, a treatment will be offered that may help to fight this disease in future patients. Because the treatment is experimental, you may not derive any direct benefit from it. The purpose of this study is to determine whether this new treatment will help to prevent the weakening of the immune defense system in the body. Because this is a new, experimental treatment, we will also be observing you to determine the side effects of the therapy. We will also monitor the effects of this treatment on cells of your immune system.

By using techniques in the laboratory, it is now possible to prepare and modify human DNA or genetic material which can be transferred to your cells in the laboratory. We will genetically modify cells which are the target of HIV infection, called T cells. We have prepared a gene delivery vehicle, either DNA or a viral vector, which contains an altered form of an AIDS virus gene, called Rev M10. When Rev M10 is expressed in lymphocytes in the laboratory, it provides protection against AIDS virus infection. This protein causes the cells which will contain it to block growth of the virus. The purpose of our study is to determine whether this gene therapy treatment will prolong the survival of T cells which contain this protective gene in your body.

Alternative Therapies

There are no known cures for patients with your disease. Other alternative treatments available to you include medication, such as AZT or ddI, to inhibit viral replication. Medical, surgical, or radiation treatment can be used on occasion to treat reversible complications. Other experimental treatments are under investigation which attempt to inhibit virus growth or to stimulate your immune system to reject your tumor, and you can be referred to physicians who are conducting such trials. In some cases, proteins are injected which can stimulate the immune system. You also have the option to receive no treatment at this time.

Procedures

Before receiving this treatment, you will receive many tests to see if you qualify for this study. These tests will be made either as an inpatient or an outpatient. These tests include: 1) blood tests 2) x-rays of the chest and 3) a blood test for the antibodies to Human Immunodeficiency Virus (HIV), which causes AIDS.

If you qualify for this study, we will take a blood sample from you, isolate your T cells, and genetically modify them in the laboratory. These cells will then be returned to you through a tube connected to a needle inserted into your vein. At different times in the protocol, blood samples will be taken, and the survival of these genetically modified cells will be assessed. This procedure will be performed prior to treatment and at intervals of 2 weeks up to 1 year.

RISKS AND SIDE EFFECTS

There are potential side effects and risks to this procedure. First, you may experience mild discomfort from needle injections. You may have mild discomfort from intravenous infusions. Second, even though the DNA inserted into your lymphocytes is considered harmless to you, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and we do not know whether cells could become abnormal after long periods of time. In animal studies, we have not observed the development of cancer cells in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other

antibiotics that are not inactivated will be available and effective in treating any potential bacterial infections.

We emphasize that this gene has never been used before in human patients. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur. Blood and possibly tissue specimens will be taken where possible to follow the duration and effects of Rev M10 gene expression.

Follow-Up

After you receive the treatment, you will be discharged from the hospital if you have no other significant medical problems. You will be required to return to the University of Michigan Medical Center for follow-up studies described above. Tests used to decide if you have responded to the therapy will be similar to those you had before beginning the therapy. If your disease progresses during treatment in this protocol, you will be eligible for other protocols and will receive treatment as indicated by your disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause your condition to deteriorate could be encountered. You will be closely monitored for such side effects.

Treatment will continue as long as there is sufficient possibility of response to warrant the risks and side effects encountered. Your physicians feel that the risks of your disease are much greater than the risks of the treatment as outlined above. Furthermore, your physicians have considered your individual situation and have concluded that, at this time, no other therapeutic approaches, such as drug treatments, are clinically indicated as being more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with you because this study does not preclude their use.

Other Pertinent Information

1. Confidentiality. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study records. This procedure may attract attention from the media. We will make every effort to protect your confidentiality. Because of media interest, however, there is a significant chance that information concerning you and your treatment will appear publicly without your consent.

2. Policy regarding research-related injuries. In the unlikely event of physical injury resulting from research procedures, the University will provide first-aid medical treatment. Treatment of injuries or side effects directly related to this experimental treatment will be provided at no cost to you. Additional medical treatment will be provided in accordance with the determination by the University of its

responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research.

3. You will not be paid to take part in this study.

4. Outpatient and inpatient studies will be ordered to determine your eligibility for study and whether you have had a response to the treatment. Clinic visits, laboratory tests, x-rays, and scans will not be free. Items which are not covered by insurance which relate to this research protocol will be covered by research grants. The cost of tests and treatments unrelated to this study will be handled as usual and will depend on whether or not you have insurance and what costs your insurance covers. Unfortunately, insurance coverage cannot be guaranteed for all tests and treatments; however, you may discuss this issue with the hospital financial office or your insurance company before you agree to participate.

5. This consent form does not include consent relating to the risks of any surgical procedures. Any surgical procedures performed will require a separate consent form.

Please understand that you are free to withdraw your consent to participate in this study at any time during treatment or follow-up and seek care from any physician with no loss of benefits or disruption in your care.

The team of physicians and nurses providing care in this study are very experienced. You must realize, however, that unforeseeable or unexplained risks are always possible when investigational therapies are undertaken.

If you have questions pertinent to this research, you should contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

If you feel that you have a research-related injury, contact Gary J. Nabel, M.D., Ph.D., at 313/747-4798.

The following numbers are for your use if medical problems develop during treatment:

Office:	313/747-4798
Clinical Research Center:	313/936-8090
Voice Mailbox:	313/764-9121 (after office hours)
Doctor or Nurse:	313/936-6266; Paul Watkins, M.D. (This is a hospital beeper; ask the paging operator to page #9128. Use the following numbers in case of an **Emergency Only**). (Outside of regular office hours if the paging service won't do.)
Home:	313/995-5848; Dr. Gary J. Nabel

Questions on my rights as a patient may be directed to Ann Munro in the Patient/Staff Relations Office at 313/763-5456.

I have fully explained to the patient, _____, the nature of the treatment program described above and such risks as are involved in its performance.

_____
Physician's Signature

I have been fully informed as to the procedures to be followed including those which are investigational, and have been given a description of the attendant discomforts, risk, and benefits to be expected, and the appropriate alternative procedures. I realize that, since my participation is voluntary, I can refuse this treatment without in any way prejudicing my future medical care. In signing this consent form, I agree to this method of treatment, and I understand that I will receive the best supportive care even if not receiving this protocol treatment. I also understand that my doctors can stop my treatment on this protocol if they feel the risks in my case have increased, over time, to exceed the potential benefits to me. I understand, also, that if I have any questions at any time, they will be answered. I have received a copy of this consent form.

I am not and will not become pregnant during this study.

I understand that the University will provide first-aid medical treatment in the unlikely event of physical injury resulting from research procedures. Treatment of injuries or side effects directly related to the experimental treatment will be provided at no cost to me. Additional medical treatment will be provided in accordance with the University's determination of its responsibility to do so. The University does not, however, provide compensation to a person who is injured while participating as a subject in research.

I have not engaged in any other research projects within the past six (6) months [ ].

Within the past six (6) months, I have been involved in a study by Dr. _____.

I have [ ] have not [ ] been under the care of a physician within the past twelve (12) months.

_____
Signature of Patient

_____
Date

_____
Witness

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Gary J. Nabel, M.D., Ph.D.	Associate Professor Internal Med. & Biological Chemistry

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Harvard College, Boston, MA	B.A.	1975	Biochemistry
Harvard University, Boston, MA	Ph.D.	1980	Cell & Dev. Bio.
Harvard Medical School, Boston, MA	M.D.	1982	Medicine
Brigham & Women's Hospital, Boston, MA	Resident	1985	Internal Medicine
Whitehead Institute, MIT, Cambridge, MA	Fellow	1987	Molecular Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE

1976-1980 Member, Board of Freshman Proctors and Advisors, Harvard College
1976-1981 Officer of Harvard University, Boston, MA
1980-1981 Instructor, Biology 99, Harvard University, Boston, MA
1980-1983 Resident Tutor in Biology, Dunster House, Harvard University, Boston
1980-1984 Fellow, Laboratory of Immunopathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
1983-1985 Clinical Fellow in Medicine, Harvard University, Boston, MA
1983-1985 Intern and Resident, Internal Medicine, Brigham and Women's Hospital, Boston, MA
1984-1987 Instructor, Harvard Medical School, Boston, MA
1985-1987 Associate, Howard Hughes Medical Institute, Whitehead Institute, MIT, Laboratory of David Baltimore
1985-1987 Associate Physician, Brigham and Women's Hospital, Boston, MA
1987-1990 Assistant Professor, Internal Medicine and Biological Chemistry, University of Michigan Medical Center, Ann Arbor, MI
1987-1991 Assistant Investigator, Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, MI
1990-present Associate Professor, Internal Medicine (with tenure) and Biological Chemistry, University of Michigan Medical Center, Ann Arbor, MI
1991-present Associate Investigator, Howard Hughes Medical Institute, University of Michigan Medical Center, Ann Arbor, MI

HONORS AND AWARDS

1971-1975 Harvard National Scholar
1975 Magna Cum Laude, Harvard College
1975 Mallinkrodt Book Prize
1976-1982 Harvard Graduate National Scholar
1982 James Tolbert Shipley Prize for Research, Harvard Medical School
1992 Off. Citation, CT State Gen. Assembly for Contributions to Hum. Gene Therapy
1992 Midwest AFCR Young Investigator Award
1992-present American Society for Clinical Investigation

PUBLICATIONS (24 of 91 total publications)

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9306-050 Raffel, Corey, Childrens Hospital Los Angeles, Los Angeles, California, and Culver, Kenneth, Iowa Methodist Medical Center, Des Moines, Iowa; *Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene.*

Date of RAC Approval: 6/8/1993
Date of NIH Approval: 9/3/1993

PROJECT TITLE: Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Thymidine Kinase Gene/Ganciclovir System.

PRINCIPAL INVESTIGATORS: Corey Raffel, M.D., Ph.D. and Kenneth W. Culver, M.D.

SCIENTIFIC ABSTRACT

Murine retroviral vectors can infect a wide variety of proliferating mammalian cell types (e.g. lymphocytes). Non-proliferating tissues (e.g. neurons) are not transduced by murine retroviral vectors. These findings suggest that this type of vector may be useful for the selective introduction of genes into growing tumors in the brain, since the tumor is essentially the only tissue that will integrate and express the vector genes.

The possibility of direct in vivo gene transfer into growing brain tumors in animals has been investigated. Rats with a malignant brain tumor were given an intratumoral stereotaxic injection of murine retroviral vector-producer cells (VPC) that were producing vectors containing the Herpes simplex-thymidine kinase (HS-tk) gene or a control VPC line containing the B-galactosidase gene. The animals were rested 5 days to allow time for the HS-tk retroviral vectors that were produced in situ to transduce the neighboring proliferating gliosarcoma. The animals were then treated with the anti-herpes drug ganciclovir (GCV). Tumors injected with the HS-tk VPC regressed completely with GCV therapy while the tumors injected with B-galactosidase VPC developed large tumors. Staining for B-galactosidase (+) cells in control animal brain revealed transduction of 10-50% of the tumor cells without evidence of transduction of the surrounding normal brain tissue. No significant toxicity was observed in toxicity studies in mice, rats and non-human primates.

Based upon these findings, we propose a human clinical trial to determine if the direct implantation of the G1TkSvNa VPC line into growing human brain tumors will induce regression with GCV therapy. The patient population primarily consists of children with recurrent malignant tumors, who are currently considered ineligible for the approved adult protocols. The RAC has previously approved two trials involving this type of therapy. In this protocol, children will undergo treatment based on the surgical accessibility of their tumor. Those with resectable tumors will undergo surgical debulking of the tumor followed by repeated treatments of HS-tk VPC into the tumor bed in an attempt to induce complete regression of the tumor with GCV therapy. Children with unresectable tumors will undergo stereotaxic injection of the tumor with VPC. These children will have failed standard therapy for their tumor and are expected to survive for several weeks to a few months.

PROJECT TITLE: Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Thymidine Kinase Gene/Ganciclovir System.

PRINCIPAL INVESTIGATORS: Corey Raffel, M.D., Ph.D. and Kenneth W. Culver, M.D.

NON-TECHNICAL ABSTRACT

The possibility of transferring a "sensitivity" gene into a growing brain tumor has been investigated. The purpose is to make the tumor sensitive to a type of chemotherapy that is relatively non-toxic to the rest of the body. The gene selected is the Herpes Simplex-thymidine kinase (HS-tk) gene. Herpes Simplex is a virus that can be killed by a drug called ganciclovir (GCV). By transferring the HS-tk gene into the tumor, using a disabled mouse virus called a "vector", the tumor will become like a herpes virus and the tumor can be killed with GCV.

Experiments in rats have shown that the direct injection of mouse cells producing a HS-tk vector into a growing brain tumor can result in complete destruction of the tumor with GCV therapy. We found no evidence of spread of the virus to the normal brain tissue or to other parts of the body. Based upon these findings, an initial trial is underway at the National Institutes of Health (NIH). To date eight people have been treated without evidence of toxicity. A second trial in adults has been approved. We now propose a human clinical trial for pediatric patients who are currently ineligible for treatment in the adult trials. In this study children with recurrent malignant brain tumors will be treated by injection of HS-tk vector-producer cells (VPC) into their tumors in an attempt to induce regression of the tumor with GCV therapy. The patient population consists of children who have failed standard therapy and have recurrent primary brain tumors with an expected survival of weeks to a few months.

CLINICAL RESEARCH PROJECT Date: 4 January, 1993

TO: Recombinant DNA Advisory Committee

Project Title: **Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with <u>in vivo</u> Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/ Ganciclovir System**

Identifying Words: gene transfer, retrovirus, pediatric brain tumor, malignant astrocytoma

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Subject of Study	Number	Sex	Range
Patients	30	M or F	2-18 years

Project Uses Ionizing Radiation: ____ Yes ✓ No

Multi-institutional Project: ____ Yes ✓ No (Approvals secured)

1. SPECIFIC AIMS

To apply gene therapy to intracranial tumors we propose a method for in vivo gene transfer of the Herpes simplex thymidine kinase (HS-tk) gene using G1TkSvNa.29 vector-producing cells. Insertion of the HS-tk gene confers a sensitivity to the anti-Herpes drug ganciclovir (GCV). The direct injection of HS-tk vector-producer cells (VPC) into growing tumors in animals can result in the complete destruction of the tumor with GCV therapy. This selective destruction of growing tumors in situ is thought to result from the transfer of the HS-tk gene into the surrounding tumor cells where the production of toxic GCV metabolites within the tumors result from the interaction of HS-tk and GCV. This procedure can result in the cure of some experimental animals with limited toxicity due to selective gene transfer into tumors. Therefore we propose to apply this technique for the treatment of recurrent primary pediatric brain tumors.

This clinical trial will establish the efficacy of single and repeated treatment of pediatric brain tumors with HS-tk VPC. Pediatric patients (ages 2-18 years) with recurrent malignant supratentorial astrocytomas will be evaluated for extent and location of their recurrence. Patients will be divided into two groups, those with resectable tumors and those with unresectable tumors, based on the location of the tumor and involvement of critical cerebral structures. Patients with resectable tumors will undergo maximal tumor resection. The tumor bed will then be injected with HS-tk VPC under direct visualization. An Ommaya reservoir will be placed in the tumor cavity. Seven days later, additional VPC will be injected into the resection cavity via the Ommaya reservoir. One week later, GCV will be administered at 5 mg/kg/dose IV BID for 14 days. The patient will receive additional cycles of VPC via the Ommaya reservoir followed by GCV depending on the tumor response and toxicities observed. Patients with unresectable tumors will undergo stereotaxic injection of HS-tk VPC directly into their tumor, followed 14 days later by treatment with GCV. If an initial response is seen and residual tumor remains with a cystic necrotic center, an Ommaya reservoir will be placed into the cyst using stereotaxic guidance and VPC will be instilled into the cyst via the reservoir. Again, GCV therapy will begin 14 days later. Additional cycles of VPC injection and GCV treatment will be considered, based on the tumor response and toxicity observed. Throughout treatment, patients will be carefully followed with serial MR scan to assess tumor response.

The major differences between this protocol versus the RAC and FDA approved protocols is the ages of patients treated and in the surgical technique. Therefore, this protocol contains several minor modifications of the previously approved protocols. However, we felt it most appropriate to present a new protocol for RAC consideration primarily because it will be conducted in children at a new institution. The NIH protocol focuses on adult patients with the use of a single stereotaxic injection of VPC into the tumor. This protocol is aimed at pediatric patients and will allow for repeated treatments via an Ommaya reservoir, similar to the Iowa protocol. Patients with large tumors or those with partial response to the initial cycle of therapy will likely benefit from repeated cycles to remaining viable tumor. Because large tumors may have a significant component of non-cycling cells, repeated

exposure to VPC and GCV will allow for tumor kill as tumor cells are recruited into the cycling pool. This proposal, utilizing surgical debulking and the repeated administration of VPC when necessary, allows for the treatment of all tumors, regardless of size.

Overall Aims and Objectives:

The study was designed to provide information on these aspects of this therapy:

1. What is the short term efficacy and toxicity of a single treatment of pediatric malignant astrocytomas with VPC and GCV?
2. Is efficacy further increased by the repeated treatment of these tumors with VPC instilled via an Ommaya reservoir?
3. What is the long term toxicity of treatment with VPC and GCV?
4. Does the patient develop a systemic immune response to the VPC as the result of injection of murine VPC?

Background and Significance

1. Pediatric malignant astrocytomas:

Brain tumors are the most common solid tumor occurring in the pediatric age group, averaging 2-5 cases per 100,000 per year (1). Astrocytomas account for about 50% of these tumors, and malignant astrocytomas of the supratentorial space account for about 25% of these (2). Despite aggressive therapy including maximal surgical resection followed by adjuvant radiation therapy and/or chemotherapy, 5 years survival is less than 30% and ten year survival is less than 10 % (3). In addition, the pediatric brain does not do well after radiation therapy A significant portion of patients treated with this modality are significantly disabled with moderate to severe developmental delay (4). When the tumors recur after therapy, the outcome is invariable fatal within weeks to a few months.

I. Selection of Patients

A. Inclusion Criteria

1. Children with recurrent malignant supratentorial astrocytomas who have failed standard therapy for their disease will be eligible to enter the study.
2. Patients will be divided into two groups based on their pretreatment MR scans. Patients will be divided into those with and without surgically resectable lesions. This decision will be made by the PIs in accordance with the standards of care of neurosurgical practice.
3. Male or female patients from 2 to 18 years of age. Patients of child bearing potential must practice strict birth control to prevent pregnancy for the duration of the study. Men will be advised to use barrier protection for the duration of the study.
4. Parents must possess the ability to give informed consent and express a willingness to meet all of the requirements of the protocol for the duration of the study. The patient will be included in the decision making process depending on age and

maturity in the opinion of the parents and PIs. All patients 12 years and older will give assent.

Recently published work suggests the feasibility of in vivo HS-tk gene transfer for the treatment of malignant astrocytomas (5). The central nervous system has several advantages of safety and efficacy for retroviral mediated in vivo gene transfer. First, retroviral vectors only integrate and therefore express vector genes proliferating cells. In the brain, the tumor contains the most mitotically active cells, with only macrophage-derived cells, blood cells and endothelial cells at minimal risk of transduction. In humans, all mitotic activity leading to the generation of neurons has ceased by birth, with the exception of the external granular cell layer of the cerebellum. By 2 years of age, all such mitoses are complete. Therefore, the possibility of specific transduction of the tumor is enhanced. Second, the brain is a partially immunologic privileged site, which may allow a somewhat longer survival of the xenogeneic murine cells in the brain and a greater transduction frequency of the growing tumor. This feature is further increased since human gliomas are known to further depress local immunity. This is thought to be secondary to a down regulation of IL-2 secretion and diminished expression of high affinity IL-2 receptors on T-lymphocytes (6). The murine cells should survive sufficiently long in the brain to allow for transduction of greater numbers of tumor cells. However, this period of survival will be limited since all cells that integrate and express HS-tk will be destroyed by the GCV or eventually destroyed by the immune system.

II. **Retroviral-mediated Gene Transfer** (7, 8)

In contrast to chemical and physical cellular transfection methods, murine retroviral vectors have proven to be extremely efficient for gene transfer into mammalian cells, with efficiencies as high as 90% in cultured murine fibroblast cell lines. Murine retroviral vectors differ from the adenoviruses and herpes simplex viruses in that the retroviruses will only integrate and subsequently express their genes in proliferating tissues (e.g. tumors). This feature of the retroviral vectors may be particularly advantageous in the brain, where the tumor is the predominant mitotic cell type, maximizing specific transduction of tumor with minimal, or absent, transduction of normal brain. The Moloney murine leukemia virus-based (MoMLV) vectors have been designed to minimize the possibility of recombination resulting in regeneration of a replication-competent virus (9,10).

In 1989, the first gene transfer experiment in humans was conducted at the NIH. This study involved the treatment of 10 patients with autologous T-cells (TIL) that have been transduced with a retroviral-vector. None of these patient have demonstrated any untoward effects secondary to receiving the genetically-altered cells (11).

Human gene therapy for adenosine deaminase deficiency has been performed on 2 children since the protocol opened in September, 1990. Twenty-three intravenous infusions of genetically altered autologous T-cells have been administered to date (12, 13).

Neither child has demonstrated any evidence of adverse effect due to the genetically altered cells.

3. Preliminary Studies

I. Pre-clinical Data

A. In Vivo Transduction with NeoR and B-galactosidase Vectors:

1. In Vivo Transduction of the NeoR Vector (LNL6) into the MCA 205 Murine Fibrosarcoma (See Appendix A: Reprint 1) (5, 15). Initial studies were performed in mice to determine if tumor cells could be transduced in vivo. The retroviral vector LNL6, which has a titer of $1.0x10^6$ cfu/ml, was utilized for these experiments. LNL6, which contains a NeoR gene promoted by the 5'-LTR, is free of replication-competent retrovirus. NeoR protects mammalian cells from the toxic effects of the neomycin analog G418. LNL6 VPC or control cells not producing vector but expressing the NeoR gene (LNL6 transduced 3T3 cells) and MCA 205 tumor were mixed in vitro and injected subcutaneously in syngeneic C57BL/6 mice. Mice were inoculated with $1x10^6$ MCA 205 tumor cells mixed with either $1x10^6$ control 3T3-LNL6 expressing cells or with $1x10^6$ LNL6 vector-producing PA317 cells. The cells were mixed just prior to subcutaneous injection. Tumors were measured twice weekly.

   To minimize the possibility of contamination of the recovered tumor cells with 3T3 and PA317 cells, we waited 4 weeks before excising the growing tumors. The excised tumors were minced and digested into a single cell suspension. Tumor cells from each group were cultured for two weeks. The cultured cells were then placed in a clonogenic assay as shown in Table 1. (Appendix A: Reprint 1). The number of colonies that grew in medium without G418 were similar in each group with a cloning efficiency of 15-20%. There were no colonies with resistance to G418 in the tumor plus control 3T3-LNL6 expressing cell group. While the tumor plus VPC group had a mean of 62% $\pm$ 15 (range 55-73) G418-resistant colonies. An assay for NPT activity, the enzyme produced by NeoR, was positive for all G418-selected tumors in the VPC group. In control mice, PA317/LNL6 producer cells alone produce a transient tumor, that is rejected in 7-10 days. Since the evaluation of tumors in the experimental group was performed at 4 weeks, it is very unlikely that these G418 resistant cells are the producer cells. The lack of G418-resistance in controls and the lack of LNL6 vector production by the recovered G418-selected cells suggests that the PA317/LN6 producer line is not responsible for the G418-resistance.
2. In Vivo Transduction of the E. coli LacZ (B-galactosidase gene) Vector(G1BgSvNa) into the 9L Rat Brain Tumor. To evaluate the in vivo transduction dynamics within brain tumors, we have used the G1BgSvNa.29 vector. G1BgSvNa.29 (produced by Genetic Therapy Inc., GTI) has a titer of $0.5\text{-}1x10^6$ cfu/ml. This vector contains NeoR and the E. coli derived gene LacZ which encodes for the production of the enzyme B-galactosidase (B-GAL) (14). The B-GAL expression can be detected using an X-GAL histochemical stain. Staining the brain with X-GAL turns B-GAL expressing cells blue. This results when an indolyl is liberated from X-GAL by the action of the B-

GAL enzyme. Subsequent oxidization and self-coupling forms an indigo blue derivative. The vector containing cells can thus be discriminated from unmodified cells and then be enumerated with light microscopy. Rats were inoculated with $4x10^4$ 9L gliosarcoma cells into the right cerebral hemisphere by stereotaxic guidance. Seven days later, $3x10^6$ PA317/G1BgSvNa VPC were injected into the tumor bearing and non-tumor bearing hemispheres using the same stereotaxic coordinates. Five, 9, and 15 days after injection of the VPC, the rats were sacrificed by an intracardiac injection of formaldehyde to fix the brain. The brains were stained with X-GAL. Control rats received G1BgSvNa.29 transduced, G418 selected 3T3 non-producer cells. The VPC and the control G1BgSvNa.29 transduced 3T3 cells were 100% positive by X-GAL staining prior to injection. In this experiment, we have shown that the injection of VPC led to transduction of 10-55% of the tumor cells in situ. Figure 2 (Appendix A: Reprint 2) presents the microscopic sections taken from the rats on day 7 and 14. The injected VPC disappeared from the injection site after day 14. There was a clear cut delineation between the transduced tumor cells and normal brain tissue. Except for the possibility of rare transduced endothelial cell in the vicinity of the tumor, no evidence of non-tumor brain transduction was evident in either cerebral hemisphere as shown in Figure 3 (Appendix A: Reprint 2). The most mitotically active endothelial cells in the area of the tumor are likely to be those responding to neovascularization signal within the tumor. Destruction of transduced endothelial cells with GCV therapy within the tumor may enhance the anti-tumor activity. The non-producer control cells did not demonstrate transduction of either brain and they were not seen in the tumor or brain after 14 days. This experiment demonstrates that local injection of a VPC line will transduce tumor cells in vivo, that the transduced tumor infiltrates surrounding brain with the non-transduced tumor and that in vivo retroviral-mediated gene transfer does not significantly effect adjacent normal non-proliferating brain tissue and that the producer cells disappear after 14 days.

B. In Vitro GCV Sensitivity of Mammalian Cells +/- Transduction with a HS-tk Vector

The G1TkSvNa.29 vector was transduced into the cell lines listed in Table 1, Appendix E in vitro using filtered supernatant from confluent producer cells. The transduced cell lines were then selected in G418 for 7 days at 1.0 mg/ml. Only cells expressing a functional NeoR gene are able to survive these G418 culture conditions producing an essentially 100% selected population of vector transduced cells. We then evaluated the sensitivity of the transduced G418-selected cells compared to the non-transduced parent cell lines. In each case, the HS-tk transduced, G418 selected cell lines were markedly more sensitive to low concentrations of GCV. Concentrations of 0.5-5 ug/ml were inhibitory to the HS-tk transduced cells in this assay. In a clonogenic assay, HS-tk positive cells were completely inhibited at 0.5 ug/ml. These findings confirm that HS-tk gene-containing retroviral vectors can effectively transduce murine and rat tumor cells and stably express both the NeoR and HS-tk genes resulting in 100% kill of the transduced cells in vitro when exposed to GCV.

All in vivo studies were done using G418-selected VPC and control non-producer cell lines that had similar patterns of GCV sensitivity conferred by a transferred HS-tk gene.

C. **Toxicity studies:**

1. Assessment of HS-tk producer toxicity in the peritoneal cavity and in the lung. We have injected PA317/HS-tk VPC and control PA317/B-GAL VPC IP into mice (Appendix C). Twelve mice received $5x10^6$ B-GAL VPC. The mice were observed for 7 days during which no evidence of toxicity was observed in either group. GCV was then administered at a dose of 150 mg/Kg BID for six days. During and after GCV administration, no toxic side effects were observed. Six mice in each group were sacrificed at the end of the GCV treatment. No gross or microscopic pathology was seen in various abdominal organs. The remaining 6 mice per group have been followed for >12 months with no evidence of long-term toxicity. Next, 12, mice each received, $5x10^5$ B-GAL or HS-tk VPC IV via the lateral tail vein to evaluate possible toxicity to the lungs where the cells are trapped. No evidence of toxicity was observed before, during and following GCV administration. Review of microscopic slides of the lungs revealed no areas of necrosis or other pathology in comparison to the control group.
2. Assessment of Transduction of Non Tumor Proliferative Tissues. Using the B-GAL gene as a marker, rat 9L brain tumors were injected with the PA317/G1BgSvNa.29 VPC. Organs were harvested and stained with X-GAL in order to estimate the frequency of vector expressing cells. Organs evaluated included the heart, lungs, liver, spleen, kidney, and small and large bowel. Organs were examined at 5,9, and 14 days following the intra-tumoral injection of the vector. Organs from rats in which control non-producer B-GAL cells were injected into their tumors served as controls. No X-GAL positive cells were seen in the heart and kidney. Occasional X-GAL positive cell were seen in the spleen, liver, and in the lungs, compatible with the distribution of normal macrophage which can produce positive results in this assay. No difference was observed between the frequency of X-GAL positive cells in these organs between the rats injected with the VPC and those injected with the non-producer cells. In normal bowel, there are a large number of X-GAL positive cells within the villi of both the small and large bowel. Again, no significant difference was observed between the non-producer and the VPC treated rats. These findings are consistent with the histology and suggest no significant spread of the vector takes place beyond the normal brain from the site of inoculation.
3. Toxicity studies of HS-tk VPC with and without GCV in normal brain. Ten rats were inoculated with $3x10^6$ PA317/HS-tk VPC into the deep white matter of the cerebral hemisphere (Appendix D). The contralateral hemisphere served as control with $3x10^6$ B-GAL-transduced 3T3 cells (3T3-B-GAL). Cells were injected in a volume of 50uL. Rats were treated with GCV at a dose of 15 mg/Kg BID for 7 days and sacrificed 3 days later for histological evaluation of the brain. The deep injection site was evident in both hemispheres with local changes secondary to the injection of cells. No difference was seen between the HS-tk VPC injection site and the B-GAL transduced 3T3 cell injection site. Surrounding brain tissue

did not show evidence of inflammation or destructive changes in either group. Dexamethasone was administered to the rats as an oral dose of 0.5 mg/Kg/day, starting on the third day post injection of the VPC. It appeared that dexamethasone pretreatment diminishes the non-specific symptoms related to the surgery and implantation of cells in the brain.

4. Toxicity studies in non-human primates. Five rhesus monkeys were used for the primate experiments. The study was designed to address 2 major issues: 1. What is the fate (survival time, proliferation potential) of the murine VPC within the brain? 2. Is there significant toxicity from the intracerebral injection of HS-tk vector-producer cells, alone or with subsequent GCV therapy?

As is in standard neurosurgical practice, the monkeys were treated with high-dose steroids (dexamethasone, 2 mg/Kg IM/day) starting 7 days before surgery and continued for 3 weeks at which point the dose was gradually tapered during one week and discontinued. Antibiotic therapy (Cefotaxime, 30 mg/Kg, IM BID) was administered starting on the day of surgery and continued for 10 days. Using general anesthesia under aseptic conditions, monkeys received stereotaxic intracerebral injection of 10^7 HS-tk VPC mixed with 10^6 B-GAL VPC (total volume of 250 uL) into the deep white matter of the right frontal lobe. One monkey received bilateral injections of an HS-tk/B-GAL vector-producer cell mixture (10:1), as described, into the right frontal lobe and 10^7 BAG-transduced 3T3 cells into the left frontal lobe. Injections were done with a 250 uL Hamilton syringe over 15 minutes.

GCV was administered to the two monkeys through a venous access port as a slow IV, infusion of 10 mg/Kg in 50 mL normal saline over 30 minutes daily for 14 days. MR scans, including T1 and T2 weighted images and gadolinium-enhanced T1 weighted studies were obtained before GCV treatment (day 5 after cell injections), 7 days after initiation of GCV therapy (14 days after cell injection), and 90 days after cell injection in non GCV-treated monkeys. Physical and neurological examinations were performed twice a day on each monkey. Repeat blood samples were obtained from all animals before and during the experiment for routine chemistry and CBC. Cerebrospinal-fluid (CSF) samples were taken by cisternal puncture from all monkeys on the tenth post-operative days and were assayed for routine chemistry and bacteriological analysis. Two monkeys who received intracerebral injections of the HS-tk producer cells without GCV have now been followed more than one year without evidence of adverse effects. These results are summarized in table 2, Appendix E.

a. Fate of the producer cells: To address this question, histologic examination was carried out on the brains of 3 monkeys. No proliferation of the VPC within the brain was observed in either the GCV-treated (2) or non-treated (1) monkeys. This was determined on the basis of histological and radiological (MR) examination of the brains. In one GCV treated animal examined 15 days after cell injection, some residual murine cells (using the B-GAL-producer cells as a marker) were found among the injected HS-tk VPC. No B-GAL positive murine cells were found in the 2 animals examined 3 weeks after cell injection, regardless if GCV was or was not administered. It is concluded that the VPC survive in the

brain up to 15 days and cannot be seen thereafter. This time frame is similar to what we have observed in the rat brain.

b. Toxicity Evaluation: Toxicity was evaluated by clinical, radiological, and laboratory (chemical, bacteriological, and histological) evaluation of the monkeys. Since the xenogeneic cells may cause an inflammatory response by leaking into the subarachnoid space (reactive meningitis), cerebrospinal fluid samples were collected from all monkeys 14 days after injection of the cells to assess that risk. All CSF samples were obtained by cisternal puncture and showed normal protein and glucose levels without pleocytosis. Bacterial cultures were negative. Table 3, Appendix E, summarizes the CSF characteristics of the samples.

c. Clinical evaluation: Neurologic examination of all monkeys, before or after cell injection and during GCV therapy was unchanged from baseline. No motor or behavioral changes were observed at any phase of the experiment. Two monkeys treated with HS-tk-producer cell injections without GCV therapy were followed for evidence of long-term toxicity. For more than 12 months following cell injection, no ill-effects or any changes from baseline neurological status have been observed.

d. Radiological evaluation - MR Studies: Both GCV-treated and non-treated monkeys' MR scans showed the localized injection site as an isointense lesion (7 days after cell injection) or hypointense lesion (7 days after start of GCV) measuring a few mm in diameter with enhancement of the surrounding rim following IV injection of gadolinium. No edema or mass effect were noted in any of the monkeys. This appearance is compatible with a local breakdown of the blood-brain barrier, as is expected from the cell injection alone.

e. Histologic Studies: Mild reactive gliosis was seen in the vicinity of the cell injection site without evidence of edema or pathological changes in neighboring brain tissue. This was true for both the GCV-treated and non-treated animals. Specimens stained for myelin showed localized demyelination limited to the injection site which did not increase in size when GCV was given. A few endothelial cells adjacent to the injection site showed B-galactosidase activity as evidence of transduction with the B-GAL vector.

f. Complications: The only complications observed were 2 septic complications related to the profound immunosuppression induced in the monkeys by high-dose steroids. One monkey (number 1), which had undergone implantation of the venous access port into the right heart developed acute bacterial endocarditis with resultant Staphylococcus aureus septicemia and septic shock that led to its death 3 days after cessation of the prophylactic antibiotic therapy. This occurred 7 days after starting GCV therapy. An MR study that was obtained 24 hours before death as well as macroscopic appearance of the brain and histology showed no evidence of CNS-related toxicity. Another monkey, which received bilateral injections (HS-tk-producer on one side and B-GAL-transduced 3T3 cells on the other side) and no GCV therapy, died on the 21st post-operative day (one day before planned sacrifice). Necropsy revealed bilateral interstitial pneumonia due to cytomegalovirus as well as systemic mycosis (Candida albicans cultured from all harvested organs) as cause of death. Again, no

clinical or histologic perturbations of the CNS were observed in that monkey.

D. In Vivo Transduction with HS-tk Vectors in Mice and Rats:

1. Anti-tumor Effect of In Situ Injection with an HS-tk Vector-producer Cell Line on the In Vivo Growth of the MCA 205 Tumor in Mice

In order to determine if in vivo gene transfer technique can promote an increased anti-tumor effect, a PA317/HS-tk producer cell line was used. PA317/HS-tk contains a NeoR gene promoted by the 5'-LTR and an SV40 promoted HS-tk gene. Figure 1 (Appendix A: Reprint 1) depicts the effect of GCV on tumor growth in vivo in mice that were injected with $1x10^6$ MCA 205 tumor cells plus $2x10^6$ 3T3-NeoR cells, NeoR VPC, 3T3/HS-tk or PA317 HS-tk VPC. The mice were ear tagged, cages coded and the tumors were measured twice weekly with a calipers in 3 dimensions. Tumor size is expressed as a volume (length x width x height). GCV treatment was initiated 4 days after injection of the cells and continued twice daily for 12 doses of 150mg/kg/dose.

All tumors grew well without GCV treatment. The growth of those tumors injected with 3T3-NeoR or NeoR VPC were not affected by GCV treatment. Tumor mixed with non-producer 3T3/HS-tk cells grew somewhat slower than the 3T3-NeoR cells during GCV treatment. However, tumors mixed with PA317/HS-tk VPC regress completely during GCV treatment. These findings suggest that the transfer of the HS-tk gene by in vivo gene transfer can eradicate tumors in vivo. Injection of transduced cells alone is insufficient for tumor eradication.

2. Anti-tumor Effect of HS-tk Vector-producer Cell Line Implantation into Growing 9L Gliosarcoma in Rats

9L is a rat gliosarcoma cell line derived from the Fisher 344 strain. This brain tumor model has been well characterized (15). Injection of $4x10^4$ 9L tumor cells into the cerebral white matter of a rat results in 100% lethality by 4 weeks. Therefore, we have used this brain tumor model to evaluate in vivo HS-tk transduction and the subsequent anti-tumor response to GCV treatment. Fisher 344 male rats weighing 250-350 grams were anesthetized and placed in a stereotaxic apparatus. On day 0, we implanted $4x10^4$ 9L tumor cells in a volume of 50 uL into the right cerebral hemisphere. 5 days later, the same stereotaxic coordinates were used to introduce either saline or $3x10^6$ PA317/HS-tk producer cells in 50 uL directly into the growing tumor. Five days later, the rats began treatment with GCV at 150mg/kg/dose twice daily. On the 5th day (10 days since inoculation of the tumor), the rats brains were examined for the extent of tumor growth. The rats treated with the HS-tk VPC and GCV are the only animals that experienced complete macroscopic elimination of the tumor (14 of 14 rats) (Appendix A: Reprint 1. Figure 2). Microscopic analysis revealed either no evidence of tumor (11 of 14 rats) or some residual, mostly necrotic, tumor in the tumor bed (3 of 14 rats). There was no evidence of vasculitis or destruction of normal tissues due to spread of the vector. This experiment further supports the data obtained with the G1NaSvBg.29 vector, that this in vivo transduction method appears to be without significant side effects and has substantial efficacy (5).

E. Bystander Effect: One of the unique features of the tumor

rejection in the HS-tk system in mice is the observation that not all the tumor's cells must contain the inserted gene in order to be killed by GCV. In mice given a subcutaneous tumor in which 100% of the cells carry the HS-tk gene, complete tumor regressions were seen in 13 of 15 animals following GCV treatment. Interestingly, when tumors established from cell mixtures containing 50% HS-tk-gene-modified cells mixed with 50% wild-type unmodified tumor cells were treated with GCV, almost all tumors regressed. Even in situations where the mixed tumor contained 90% unmodified, wild-type tumor cells mixed with only 10% HS-tk modified tumor cells, complete regression of the cancer was observed with GCV treatment in 9 of 15 animals (Table 2, Appendix A: Reprint 1).

The mechanism of this "bystander tumor kill" is not yet completely understood. This may involve the production of toxic triphosphates produced by the interaction of thymidine kinase and GCV within the tumor leading to inhibition of DNA synthesis and death of replicating cells. This does not seem to involve generalized non-specific cellular toxicity since the overlying skin and other tissues surrounding these HS-tk treated tumors are not injured while the tumors expressing the genes and the admixed wild-type tumor cells are completely destroyed.

The 9L rat gliosarcoma and the human glioblastoma cell lines U251 and A172 were also sensitive to this "bystander" effect in an in vitro mixing experiment. G1TkSvNa.29 transduced and non-transduced tumor cells were mixed at different ratios in 96 well microtiter dishes. GCV was added to the wells and 24 or 48 hours later, the cultures were pulsed with tritiated thymidine. These bar graphs depict a greater decrease in proliferation than would be expected at a GCV level of 5.1 ug/ml in the medium (the numbers over the bars represent the percent of transduced tumor in the culture) (Appendix B: Figure 1). The 5.1 ug/ml concentration is easily within the therapeutic range established in humans with a dose of 5 mg/kg/dose. Since it is unlikely that 100% of the tumor cells in the brains of our patients will be successfully gene-modified, this "bystander" effect is very important for the successful elimination of the tumor using this approach (See Appendix B: Figure 1).

F. Effects of Ommaya Reservoir on VPC.

In Vitro: In order to determine if the Ommaya reservoir would bind significant numbers of VPC or induce cell clumping, 5 mls of PA317/G1TkSvNa VPC's were passed through an Ommaya reservoir with a 5% loss of cells without visual evidence of cell clumping.

In Vivo: In order to determine if the Ommaya reservoir would inhibit in vivo gene transfer, G1TkSvNa.29 VPC's which were passed through an Ommaya, mixed (3:1) with the MCA 205 fibrosarcoma and injected SQ into C57BL/6 syngeneic mice. Control animals were injected with mixtures of G1TkSvNa.29 VPC's and MCA 205 that were taken directly from culture or injected with mixtures of G1BgSvNa.29 VPC's and MCA 205 tumor cells. GCV treatment was initiated 9 days after injection of the cells and continued twice daily for 14 doses of 150mg/kg/dose.

All tumor/VPC mixtures grew well without GCV treatment. The growth of those tumors injected with mixtures of G1BgSvNa.29 VPC and MCA 205 tumors were not affected by GCV treatment. All five mice in each G1TkSvNa.29 VPC/205 tumor mixture group whether passed

through the Ommaya reservoir or taken directly from culture showed tumor regression with GCV treatment. These findings suggest that the passage of VPC's through the Ommaya reservoir had no adverse effect on their ability to transduce tumor in vivo, and sensitize them to GCV.

G. Effects of CSF and serum on in vitro gene transfer.

CSF was obtained from 3 patients with CNS malignancies. The CSF was placed on ice, spun and frozen. Serum was collected from a peripheral vein, placed on ice, spun and frozen.

The human U251 glioblastoma cell line was placed at 50,000 cells per well in 6 well plates. Fresh G1BgSvNa.29 supernate was harvest from a confluent T-175 flask. 1:1 and 1:10 mixtures of CSF (with or without heating for 30 minutes at 56^{0}C) to supernate were placed on the U251 cells after aspiration of culture medium. Serum was added at 1:4 or a 1:20 dilution (with or without heating for 30 minutes at 56^{0}C) to supernate and placed on the U251 cells after aspiration of culture medium. Protamine (10u/ml) was added to each well. After an 8 hour incubation, the supernate mixture was removed and replaced with cell culture medium. The following day, the cells were washed, fixed with formalin/gluteraldehyde and stained with X-GAL. The percent of cells staining blue was enumerated with light microscopy. The U251 cell line has no detectable background staining using this technique (see Appendix E: table 4).

H. Pre-Clinical Summary: The pre-clinical findings noted above suggest that the direct inoculation of a brain tumor with HS-tk retroviral vector-producing cells can mediate complete tumor rejection in animals. This in vivo gene transfer methodology selectively alters the sensitivity of a tumor cell to systemic chemotherapy. Treatment with GCV does not result in widespread damage to the host immune system like many forms of chemotherapy. Our animal studies have demonstrated no significant toxicity to normal brain or any of the proliferating non-CNS tissues, suggesting that the implantation of VPC in a brain tumor is not associated with non-specific systemic toxicity.

II. Clinical Data

Eight patients have been treated with the stereotaxic injection of HS-tk VPC and GCV infusion. No evidence of toxicity due to cell implantation or GCV administration has been observed as of April 1, 1993.

4. Research Design and Methods

I. Selection of Patients

A. Inclusion Criteria

1. Children with malignant supratentorial astrocytomas who failed standard therapy for their disease will be eligible to enter the study.
2. Tumors will be divided into surgically resectable and surgically unresectable, as estimated from the pre-treatment radiological evaluation. These decisions will be made by the PIs in accordance with the standards of care of neurosurgical practice.
3. Male or non-pregnant female patients 2-18 years of age. Patients of child bearing potential must practice strict birth

control to prevent pregnancy for the duration of the study. Men will be advised to use barrier protection for the duration of the study.

4. Parents must possess the ability to give informed consent and express a willingness to meet all the expected requirements of the protocol for the duration of the study. (Appendix L).

5. Adequate baseline organ function as assessed by the following laboratory values before surgery:

   a. Adequate renal function with serum creatinine of $\leq$ 1.5 mg/dl or creatinine clearance $\geq$ 45 $mL/min/m^2$.
   b. Platelet count $\geq$ 100,000 $platelets/mm^3$.
   c. Absolute neutrophil count $\geq$ $1000/mm^3$.
   d. Hemoglobin $\geq$ 8.5 mg/dL.
   e. Normal Partial Thromboplastin Time (PTT), and Pro Thrombin Time (PT).
   f. Bilirubin $\leq$ 2.5 mg/dL, SGOT and SGPT < 4x normal.
   g. Patients with a performance status of $\leq$ ECOG 3 will be excluded from the study. (See Appendix H).

6. Patients admitted for study under this protocol will be registered with the principal investigator at Childrens Hospital Los Angeles.

B. Exclusion Criteria:

Patients with the following conditions will be excluded from the study:

1. Acute infection. Active infection is defined as any acute viral, bacterial or fungal infection which requires specific therapy.
2. Neurological deterioration. Patients with an increased ICP who require prompt reduction of ICP will be excluded.
3. HIV positive patients.
4. Pregnant patients.
5. Multiple tumors.
6. Post-surgical cavity in continuity with subarachnoid cistern or ventricular space.
7. Prior history of brachytherapy or stereotaxic radiotherapy.
8. Tumor greater than 7 cm. in any diameter.

II. Clinical Evaluation

A. Preliminary Evaluation and Screening

The following evaluation must be completed before patient entry into the study.

1. Before a patient can be entered into this study, the investigators must receive information from the referring physician(s) detailing the clinical history, general laboratory results, specific neurologic and radiologic evaluations, their diagnosis, and all previous therapies (Appendix I: Patient Screening Form).
2. Data substantiating the histopathological diagnosis must be received and reviewed by the reference pathologist (Dr. Floyd Gilles, CHLA) prior to participation in the study. If no recent tissue diagnosis is available, the nature of the space occupying lesion (e.g. tumor vs. radiation necrosis) will be determined by a stereotaxic

biopsy of the lesion prior to cell injection.

3. Pretreatment general physical examination and a comprehensive neurologic evaluation (Screening).
4. HIV test (must be negative) (Screening).
5. B-HCG a pregnancy test must be negative within 14 days of surgery in children of child bearing potential.
6. Renal and hepatic blood chemistries (uric acid, calcium, phosphorus, magnesium, SGOT, SGPT, Alkaline Phosphatase, LHD, total bilirubin, BUN, creatinine, albumin, total protein, amylase, electrolytes, glucose) (Screening).
7. Urinalysis (Screening)
8. PT, PTT, fibrinogen (Screening)
9. CBC with differential and platelets (Screening).
10. Radiological Evaluation. Each patient will undergo an MR and/or CT study of the brain with contrast enhancement. Preliminary decisions regarding the eligibility of the patient for treatment, and the injections and/or procedures needed for each patient will be decided based on these studies (Screening).
11. CXR
12. Freeze baseline serum and peripheral blood mononuclear cells to allow testing for antibody and T-cell reactivity to PA317 cells.

B. Evaluations during the treatment period

1. Physical examination to include neurologic examination will be performed at each patient visit. Patients will be evaluated weekly at a minimum throughout the treatment period.
2. MR scans of the brain will be obtained without 48 hours after surgery and approximately 1 week after completion of each course of GCV therapy. Standard neurosurgical follow-up will be employed. CT scan may also be obtained if indicated.
3. Creatinine clearance tests, liver function tests, CBC, coagulation studies and blood chemistry will be monitored as indicated.

C. Evaluation following the treatment period.

1. Patients will be seen as out-patients at 2 week intervals for the first 2 months and on a monthly basis for one year. During the subsequent year patients will be followed at two month intervals.

    a. Laboratory tests as required for standard neurosurgical care.
    b. Physical examination with a detailed neurologic exam.
    c. MR scan will be obtained every 4-8 weeks. These scans will be used to assess the status of the patient's tumor. A patient will be considered to have failed therapy if there is evidence of tumor growth on a follow-up scan.
    d. B-HCG a pregnancy test will be repeated monthly for women with child bearing potential while on therapy.
    e. Radiological evaluation: Patients will have a CT scan or MR scan of the brain (if needed) with contrast enhancement if appropriate.
    f. Freeze serum and peripheral blood mononuclear cells.

2. Yearly follow-up laboratory evaluation. Patients will be requested to be followed once a year for the rest of their life for retroviral gene transfer safety monitoring.
    a. CBC with Differential count
    b. Serum for antibody to PA317 cells.
    c. PCR on peripheral blood mononuclear cell DNA for vector sequences.
    d. Western blot analysis of serum for antibody to retroviral antigens.
    e. if at any time a new malignancy develops, we shall attempt to obtain involved tissue for analysis for the vector DNA.
3. It is understood that the performance of an individual study or test as specified in this protocol is subject to factors such as patient compliance, scheduling difficulties, equipment malfunction, or the clinical judgment of the principal investigator or patient care physician, and that a test may not be done in an individual instance with no violation of the protocol. However, any systematic modification of the original protocol in this regard, whether related to patient safety or not, will be submitted to the IRB for approval.
4. Autopsy: An attempt will be made to perform a complete autopsy on any patient who dies during the study. Whenever possible, tissues from the brain, tumor(s) and/or bone marrow, will be evaluated for presence of the G1TkSvNa.29 vector by PCR.

D. Criteria for Response

1) Non responders:	Less than 25% decrease or increase in the bidirectional diameter of the tumor by CT scan and/or MR scan.
2) Minimal responders:	25-49% decrease in tumor size defined by the greatest bidirectional diameter by MR or CT scan.
2) Partial responders:	≥50% decrease in tumor size by CT scan and/or MR scan defined by the greatest bidirectional diameter by MR or CT scan.
3) Complete responder:	No remaining tumor on CT scan and/or MR at 3 months of 4 weeks duration.

E. Re-Treatment Criteria

Patients who demonstrate one of the following responses to therapy will be considered for re-treatment with three 5 week cycles of VPC injection through the Ommaya reservoir.

1). Patients who achieve a minimal, partial or complete response.
2). Patients who achieve a complete response and then show new evidence of disease progression in the same location of the original resection.
3). Patients who have stable disease for at least 4 months following the initial surgery.
4). Patients who had no evaluable disease on the post-operative CT or MR scan, but later show evidence of progressive disease.

F. Off-Treatment Criteria

Criteria for going off therapy: The major criterion for suspending enrollment of patients into the protocol would be evidence of

significant toxicity that is directly related to the application of our experimental therapy. Such toxicity may include the following:

a. Irreversible diffuse cerebral dysfunction (motor, sensory, cognitive).
b. Irreversible regional cerebral injury that is not related and cannot be explained by the physical aspects of the surgical procedures alone.
c. Permanent Grade 3 toxicity or recurrent Grade 4 toxicity as specified in Appendix J of the protocol. It should be noted that the assessment of neurological toxicity (Neurosensory, neuromotor, neurocortical, neurocerebellar, neuro-mood, neuro-headache, etc. as appears in Appendix J - Toxicity Criteria) relates to the relative changes from the pre-treatment neurological status. If such toxicity is observed in 2 of the first 3 patients, the study will be suspended and reevaluated. If no such toxicity is observed in the first 3 patients, but later is seen, the study will be abandoned.
d. Other anticipated toxic phenomena that are not related directly to the in situ transduction of the brain tumor (such as toxicity secondary to GCV therapy), or neurological complications which can be explained by the presence of the mass lesion and are compatible with the natural history of malignant astrocytomas, will not be considered a sufficient indication for the termination of the study. Any additional occurrence of toxicity will be discussed with the Chairman of the IRB.
e. Upon request of the participating patient or the person with the power of attorney the patient may withdraw from the study.
f. A patient will be considered to have failed treatment and be taken off the study if any follow-up scans shows an increase in the size of tumor after treatment.

III. Data Collection and Monitoring

A. Study Monitoring. Site visits will be done by GTI to monitor the data in the research records and to ensure that all regulatory requirements surrounding the study are met. The investigator will allow study monitors and the FDA to inspect study documents (e.g. consent forms, certificate of analysis, case report forms, and pertinent hospital or clinical charts).

B. Reporting of Adverse Reactions. (ADRs) to the IND Drug will be reported promptly to the FDA, IRB and IBC. ADR reports are required even if only a suspicion of a drug effect. Previously unknown Grade 2 or 3 reactions will be reported in writing within 10 working days. Grade 4 (life-threatening) reactions and patient deaths while on treatment will be reported by phone within 24 hours. A written report will follow within 10 working days

C. Protocol Amendment Procedures. Any revisions to the original protocol will be discussed by all investigators, the FDA and others as required. If the consensus is to revise the current

protocol, a formal list of changes will accompany the amended protocol and these will be submitted to the FDA, the site's IRB and other committees.

D. **Publications resulting from Trial.** Any manuscript, abstract, or presentation will be available to all the study investigators involved in this protocol and the sponsoring company for review prior to submission.

IV. **Nature of Procedures or Therapeutic agents.**__(See schema in Appendix F)

A. **Surgical Procedures.** For patients with unresectable tumor, VPC will be injected stereotaxically into multiple tumor sites (maximum of 5cc total volume VPC). For patients with resectable lesions a craniotomy will be performed and optimal tumor removal will be attempted (day 1). The lining of the cavity will then be infiltrated at multiple sites with the HS-tk VPC (day 1) to a maximum volume of 10 mL. VPC suspended at a concentration of $1\text{-}2X10^8$ cell/ml will be innoculated slowly in 0.1 to 0.25 inocular at sites distributed as evenly around the tumor site as possible. A diagram of the sites of the inocula and volumes of cells delivered at each site will be kept as part of the permanent research records. An Ommaya reservoir, which provides an access port to the brain, will be placed into the tumor bed.

B. **Use of the Ommaya Reservoir.** Seven days after the initial surgery, the Ommaya reservoir will have sealed into the patients brain and surrounding tissues. On day 8 the skin overlying the Ommaya will be cleansed with betadine. A needle will be inserted through the overlying skin into the reservoir with the patient awake. With the patient in the recumbent position the Ommaya reservoir will be gently irrigated with normal saline to clarify the contents of the tumor cavity. An attempt will be made to deliver no less than 5 or more than 10 cc of VPC. The administration of VPC cells through the Ommaya will be followed by a flush of normal saline equal in volume to the volume of the Ommaya delivery system. GCV will be administered starting on the 15th post-operative day for 14 days (days 15-28). Repeat administration of producer cells will be done on day 49, followed by another cycle of GCV administration 14 days later (days 63-77). Re-treatment will consist of VPC injection through the Ommaya reservoir on day 1 of the cycle, GCV will commence on day 15 to continue for 14 days (days 15-28). No treatment will be given for 7 days (days 29-35). Cycle 2 of 3 begins on day 36. A CT or MR scan will be obtained every 5 weeks during this part of the study.

C. **Volume and number of injected cells:** Factors such as tumor size, location, and the preoperative neurological condition of the patient will determine the injectable volume. The final cell concentration will be adjusted to $1\text{-}2X10^8$ cells/mL.

D. **Peri-operative medications:**

1. Antibiotics: All patients will receive a single dose of vancomicin or the equivalent just prior to the initial surgical procedure and prior to the injection of the Ommaya reservoir.
2. Steroids: All patients will receive dexamethasone at 0.05

mg/kg/q6 hours the day of each VPC injection as indicated and treatment will be continued until GCV is discontinued. Dexamethasone will then be tapered. As experience is gained, the need for dexamethasone dosing may be modified.

3. Mannitol: Patients may receive mannitol during the surgical procedure at 1g/kg and the dose repeated TID for 24 hours following the procedure, as clinically indicated.
4. Anticonvulsant: Anticonvulsive therapy will be administered according to the usual neurosurgical guidelines.
5. Analgesics: Pain medication will include Acetaminophen 15 mg/kg, Q 4 hours, to a maximum of 1000 mg/dose.

E. The G1TkSvNa.29 Retroviral Vector (Appendix G). G1TkSvNa.29 is a retroviral vector derived from the Moloney murine leukemia virus (MoMLV). This vector contains a Herpes simplex type I thymidine kinase (HS-tk) gene cDNA that is transcribed from the viral LTR and a bacterial neomycin resistance (NeoR) gene transcribed from an internal SV40 (simian virus 40) early promoter (LTR--HS-tk-SV-NeoR-LTR) in the G1 vector backbone (Genetic Therapy Inc., Gaithersburg, Md). This G1-based vector has been modified for increased safety by alteration of the gag start codon to a stop codon, and by elimination of viral sequences needed in trans for the formation of the virus particle. This has been shown to minimize the potential for the development of replication-competent virus production from producer cells which contain the vector. The HS-tk gene is a negative selectable marker or "suicide" gene. When a HS-tk transduced cell is exposed to GCV, the GCV acts as a substrate for phosphorylation by HS-tk resulting in a monophosphate (MP) form of the drug. Cellular phosphorylases converts this GCV-MP to GCV-triphosphate (GCV-TP) that inhibits DNA polymerase and is incorporated into DNA resulting in an inability of the cell to proliferate. The end result is cell death for the HS-tk transduced cells (19, 23). The NeoR gene is a positive selectable marker. The bacterial NeoR gene encodes for NPT II (neomycin phosphortransferase II), an enzyme that will protect G1TkSvNa.29 expressing cells from the toxic effects of G418 (a neomycin analog). NeoR has been used in human clinical trials to date without adverse effect.

Severe edema: Edema secondary to necrosis and destruction of the transduced tumor cells may occur. The magnitude of such edema, if it occurs at all, is unknown. As a precaution, all patients will be treated with high-dose steroids. If edema around the tumor is not responsive to therapy, permanent, significant neurological deficits or death may result.

Surgical Procedures: The surgical procedures carry a risk for loss of neurological function, non-neurological complications and death. This risk depends on the preoperative condition of the patient, size and location of the tumor, and associated disease (e.g. renal failure, etc.). The individual risk will be determined prior to the decision on the line of therapy and will be discussed with the patient prior to surgery.

B. Use of the Ommaya Reservoir. The Ommaya reservoir is a commonly used device in neurosurgery. The potential complications resulting from its use are infection and

bleeding. Every effort will be used to maintain sterility and prevent bleeding.

C. **The development of replication-competent retrovirus.** As discussed (Section IV.E.).

D. **Survival of Vector Producer Cells in the Host.** The PA317 cells can survive only 7-14 days in a subcutaneous site in syngeneic (H-2b) mice and only 7-14 days in xenogeneic rat brain. Likewise in immunosuppressed monkeys, the producer cells could not be identified > 14 days after injection. Taken together, these findings suggest that if any PA137 cells are not killed by GCV will not have the ability to survive long term in the human brain.

E. **Dissemination of G1TkSvNa.29** Our MoMLV amphotropic retroviral vectors are directly inactivated by human complement without antibody. Therefore, escape of free vector into the cerebral spinal fluid or the vascular space should result in immediate inactivation. The gene transfer in this direct injection system is most likely due to the intimate contact of the tumor and VPC. Any vector particles that are released in the area of injection will be quickly bound by the thousands of amphotropic vector receptors on each tumor cell and other host cells. Vectors binding to non-dividing cells in the brain will be lost. Even if all of the vector particles produced were able to escape direct transfer into adjacent tumor cells of binding to non-dividing cells and cross the blood-barrier, the number of vector particles relative to the number of receptors in any organ would still be very small suggesting a minimal risk of injury to proliferating cells in any non-CNS organ. Any direct transfer into neurons by cell-to-cell contact will not result in HS-tk gene integration and therefore, should not pose a risk for their destruction with GCV treatment.

F. **Transduction of surrounding brain tissue.** There is no evidence in our animal model that transduction of surrounding normal brain tissue is likely to occur (Section 3.I.A.2.). However, if the HS-tk gene is introduced into a large number of normal dividing cells within the CNS (such as endothelial cells and astroglial cells), vasculitis like symptoms (headaches, convulsions, bleeding) may develop. Such changes however will be localized to the immediate vicinity of the tumor as had been shown in our experiments (3.I.C.2).

G. **Insertional Mutagenesis.** Retroviral vector DNA is inserted randomly into the genome of proliferating cells, The random nature of this integration has the potential of an untoward insertional event. If the insertion disrupts a gene essential for maintaining cell function, that particular cell will die. Since the gene transfer will occur most predominantly in tumor cells, the vector insertion site will result in the death of a few tumor cells without GCV, which should not pose a problem. The risk of oncogenic transformation with these replication-incompetent retroviral vectors cannot be accurately estimated since that has never been a documented occurrence in animals or man. While this is a real risk, this risk must be very low, especially in this protocol, where all vector containing cells will be killed by GCV within 2-3 weeks

of injection of the producer cells. Based upon available data, the risk of death secondary to their tumor far exceeds any risk of insertional mutagenesis.

H. **Ganciclovir sodium (GCV; Cytovene).** The GCV used in this trial will be obtained from Syntex Corporation (Palo Alto, CA). GCV is an FDA approved drug for cytomegalovirus (CMV) retinitis therapy in immunocompromised individuals. The drug is given by IV infusion over 1 hour (dose of 5 mg/kg) twice daily for 14-21 days. FDA approved guidelines (where applicable), administration procedures, drug interactions, and patient monitoring will be followed. There is minimal information regarding the use of GCV for the treatment of humans as a method to destroy HS-tk gene transduced human cells. Further information about toxicity is being generated in the initial NIH trial. To date, no toxicities have been noted in the first four patients.

GCV has been noted to cross the blood-brain barrier. The cerebral spinal fluid (CSF) plasma ratio has been estimated in 3 patients at various time intervals with ratios ranging from 0.24 to 0.7 (0.31-0.68 ug/mL in the CSF and 0.44-2.20 ug/mL in the plasma). Peak plasma levels have been documented to reach 9ug/mL. These CSF and plasma levels are expected to be within the range of GCV levels needed to kill the HS-tk transduced cells based on in vitro studies (0.5 ug/mL will prevent growth of HS-tk transduced tumor cells). If the patient has evidence of renal impairment, the dose will be adjusted as suggested in the GCV monograph.

Granulocytopenia (absolute neutrophil count (ANC) of $\leq$1000 cells/mm^3) in 40% of patients and thrombocytopenia ($\leq$50,000 platelets/mm^3) in 20% are the most frequent side effects. This data was collected in CMV infected AIDS patients, who may have been more susceptible to marrow suppression, due to other opportunistic infections or drug therapy. The actual risk to our patients is unknown. Each patient will be closely monitored for the development of granulocytopenia and thrombocytopenia. The development of an ANC of <500 cells/mm^3 or a platelet count of <25,000 platelets/mm^3 will require a dose interruption or decrease until the ANC is >750 cells/mm^3 platelet count is >40,000 platelets/mm^3. Other side effects occurring in approximately 2% of patients include anemia, fever, rash and abnormal liver function. Fever and chills will be treated with acetaminophen (650 mg every 4 hours) and rash with diphenhydramine (50 mg every 6 hours). See the monograph for complete toxicity criteria. Our treatment protocol will use the known tolerated dose (10mg/kg/day) in non-infected patients for only 14 days.

I. **Statistics.** Historical data on the survival of patients following re-operation for recurrent primary brain tumors will serve as a historical control. This is a two arm study with patient's divided into groups based on the resectibility of their tumor. Because treatment is not randomized and the treatment arms are similar, we will consider this a prospective, single arm trial to determine the response rate of this therapy for statistical purposes. A therapy with a

response rate of 20% in this population of patients would be considered worthy of further evaluation. In order to have less than a 10% chance of missing such a response and less than a 10% chance of falsely determining a true response rate of 5%, the following design will be employed. Fifteen patients will be placed on a study and evaluated. If no patient demonstrates response, then the trial will be closed. If at lest 1 patient demonstrates response, then a total of 30 patients will be enrolled in this study.

J. **Early Stopping Rules.** Permanent grade 3 or recurrent grade 4 toxicity: If such toxicity is observed in 2 of the first 3 patients, the study will be suspended and re-evaluated. If no such toxicity is observed in the first 3 patients, but later is seen, the study will be abandoned.

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Medical Record: Consent to Participate in a Research Study
Childrens Hospital Los Angeles

Study Number:_____ Principal Investigator: Corey Raffel, M.D., Ph.D.

CONSENT TO PARTICIPATE IN A RESEARCH STUDY: GENE THERAPY FOR THE TREATMENT OF RECURRENT PEDIATRIC MALIGNANT ASTROCYTOMAS WITH IN VIVO TUMOR TRANSDUCTION WITH THE HERPES SIMPLEX THYMIDINE KINASE GENE/GANCICLOVIR SYSTEM

FEDERAL REGULATIONS REQUIRE WRITTEN INFORMED CONSENT FROM PARTICIPANTS IN A RESEARCH STUDY. THE NATURE AND RISKS OF PARTICIPATION MUST BE EXPLAINED. INDIVIDUALS MUST DECIDE TO PARTICIPATE OR NOT TO PARTICIPATE IN A FREE AND INFORMED MANNER. YOU ARE ASKED TO READ THE FOLLOWING MATERIAL TO ENSURE THAT YOU ARE INFORMED OF THE NATURE OF THE RESEARCH STUDY. THE REQUIREMENTS FOR PARTICIPATION IN THE STUDY ARE DESCRIBED. SIGNING THIS FORM WILL INDICATE THAT YOU HAVE BEEN INFORMED AND THAT YOU GIVE YOUR CONSENT.

BACKGROUND AND PURPOSE

Your child has a diagnosis of recurrent malignant astrocytoma and, therefore, is eligible to participate in this study program.

There is no effective treatment for malignant brain tumors in children that recur following surgery, radiation therapy and/or chemotherapy. Therefore, we have developed a new experimental approach for the treatment of recurrent brain tumors that takes advantage of knowledge gained in the fields of genetics and gene therapy. A virus that causes mouth sores and other types of infections is called Herpes simplex. It can be treated with a drug called Cytovene. The Herpes simplex virus is killed by Cytovene because the virus contains a specific gene called the Herpes-thymidine kinase gene (TK gene). The TK gene has been isolated in the laboratory and in experiments has been injected into brain tumors in rats. This causes the brain tumor cells containing the TK gene to be killed when the rats are treated with Cytovene. In some of the rats treated this way, the brain tumors disappeared. Because the TK gene is delivered to the brain tumor using a method that will only effect tumor cells and not normal brain cells, only tumor cells should be killed by Cytovene. The purpose of this study is to gain information about whether or not this new approach is effective in treating pediatric brain tumors. Although the findings in animals are encouraging, your child will be one of the first humans to receive this investigational treatment for brain tumors. While we hope to gain information on the usefulness of this approach in humans, we cannot know whether the tumor in your child will get smaller as a result of this treatment. It is possible that this treatment could make your child worse.

What is gene therapy for brain tumors?

We will attempt to change the genetic material of your child's

brain tumor cells by inserting the TK gene into the tumor cells. The way we will transport the TK gene into the tumor cells is to use a "vehicle" to carry the TK gene into the cells. An example is that if you want to move a passenger you need to put him in a car. The vehicle (or car) in this case is a different virus - a retrovirus that is found in mice - that has been inactivated so that it cannot cause disease. We call this vehicle a "vector". We insert the TK gene (the passenger) into the vector and the vector carries the gene into your child's brain tumor cells. The vector/gene combination is produced by "vector-producer" cells (little factories that make the cars containing the passengers). When the vector/gene combination gets into the tumor cells it inserts itself into the cells' genetic material (chromosomes) and tells the tumor cells to begin making TK. We will also attach to the vector vehicle a bacterial gene that we think is harmless; this gene serves to mark the location of the TK gene in your child's body. When we give your child Cytovene, the tumor cells containing the TK gene produce harmful substances within themselves that cause the tumor cells to die. We do not expect normal brain cells to be injured because the vector/gene combination is attracted only to growing cells. Normal brain cells are not growing, but tumor cells are. The tumor cells, not the normal brain cells, are targets for the vector/gene combination.

POTENTIAL BENEFITS

This study utilizes a type of therapy that has not been used previously for the treatment of pediatric brain tumors. By using this therapy on your child, we will be learning whether the therapy is effective or not. If the therapy is effective, some or all of the tumor cells in your child's brain will be killed. If some of the cells are killed, your child's life may be prolonged. If all of the cells are killed, your child may be cured. We emphasize that we do not know if this therapy will be effective. It is entirely possible that the therapy will have no effect on your child's tumor. It is possible that the therapy will make your child worse.

We believe that the information obtained from patients in this study may be of beneifit to future children with similar brain tumors.

STUDY PLAN

Pretreatment Evaluation:

Your child may be admitted to the hospital for approximately 1 week for the pretreatment evaluation and the following procedures. Your child will have a complete neurological and physical examination, blood and urine tests to determine his/her overall physical health. The amount of blood drawn from your child will be no more than 2 Tbs per day. Your child may experience some discomfort or temporary pain at the site of the needle entry for the routine blood studies. There is remote risk of fainting and local infection.

Your child will also have X-rays of the chest and head and any other areas of the body as indicated by his or her medical examination and history. Additional tests may be required depending upon his/her

state of health and prior therapies. The blood tests will also include a test for the HIV (AIDS) virus. Women of child bearing age will also have a pregnancy test. Children with HIV and pregnant women will not be eligible for this protocol. (You will be required to sign a separate consent form for the HIV blood test.)

An MR scan and/or a CT scan of your child's brain will be done. Both scans are designed to examine the changes in the nervous system that occur with the recurrence of the brain tumor. MR uses a strong magnetic field and radio waves to show structural and chemical changes in the brain tissues. A CT scan uses X-rays. The type of scan chosen will depend upon the status of the tumor and the response to this therapy. During the scan, a needle will be placed into a vein in your child's arm and an image-enhancing dye may be administered through that vein before obtaining a second set of scans. Each scan takes about 60-90 minutes. Scans will be repeated periodically after injection of the vector-producing cells to monitor the effect of this therapy on tumor growth.

If the size and location of your child's tumor are such that partial surgical removal is possible, he/she will undergo three procedures in the following order: 1. Brain surgery to remove as much of the tumor as possible with injection of vector-producing cells into areas of the remaining tumor. An Ommaya reservoir will be placed under the scalp with a tube connecting the reservoir to the tumor site. The reservoir allows for the repeated injection of vector-producing cells without repeated surgery on the brain. 2. One week after surgery, an injection of additional vector-producer cells through the Ommaya reservoir into the tumor cavity. 3. Twice daily intravenous injections of Cytovene for 14 days beginning two weeks after the initial surgery.

Surgery:

Your child will be taken to the operating room and, following administration of general anesthesia, a craniotomy (direct surgery on the brain) will be performed. A piece of skull will be removed, the coverings of the brain will be opened and as much tumor will be removed as possible. Since it is impossible to completely remove a recurrent brain tumor surgically, the remaining tumor will be injected with the vector-producing cells during the operation. Following surgery, your child will be monitored in the intensive care unit (ICU). During the various stages of therapy, your child may receive various drugs to prevent convulsions and swelling within the brain. These drugs are used routinely in neurosurgery.

Injection of vector-producing cells through the Ommaya reservoir:

Seven days after surgery, additional vector-producing cells will be injected into the area of the remaining tumor. This will be performed in the clinic by the neurosurgery staff. There is no need for anesthesia.

Cytovene administration:

Fourteen days after surgery, Cytovene infusions will be started. Cytovene will be administered into a vein by slow drip lasting about one hour. The drug will be given two times a day for 14 days. Your child may have an MR or CT scan before, during and after Cytovene therapy.

If your child's tumor is unresectable and surgery is too dangerous due to its location in the brain, then vector-producing cells will be placed directly into the tumor using stereotaxy. For this procedure, your child will be put to sleep with general anesthesia, a localizing device will be fixed to your child's skull and a CT scan with contrast injection will be obtained. Using the information from the scan, suitable sites in the tumor will be injected with vector-producing cells by passing a needle through the brain into the tumor. After this operation, monitoring and treatment with Cyovene will proceed as described above. It is possible that at some time during treatment, your child may become a candidate for placement of an Ommaya reservoir into the tumor to allow for additional treatment with vector-producing cells. This will require a second stereotaxic operation to allow for placement of the reservoir into the tumor.

Follow-up Care and Evaluation:

After your child finishes the course of Cytovene he/she will be discharged from the hospital. Two weeks after discharge, he/she will be required to return for follow-up studies. He/she will undergo neurological and physical examination and laboratory test, as were performed before surgery. An MR or CT scan of the brain may also be obtained. Following the initial visit, he/she will be seen as an outpatient at 2-8 week intervals for the first year. The number of visits after the first year will depend on the status of the tumor. We expect that we will receive one sample of blood per year for the remainder of your child's life to monitor the safety of this gene transfer methodology.

POTENTIAL RISKS

Risk of MR and CT scans:

MR and CT brain scans are painless procedures that are very safe for most patients. During the scan your child will lay flat in a long metal cylinder, which people who fear closed spaces sometimes find a little frightening. Patients can be observed at all times by the operator and can be assisted if necessary. Your child will be moved out of the machine if requested. The MR machine makes a thumping sound while operating.

A small proportion of people develop short-lived reactions during the dye administration including nausea, headache, hot flashes, and heart palpitations. These symptoms usually resolve spontaneously within minutes. A smaller group of patients are actually allergic to the dye and develop a rash, hives, respiratory difficulty, and in extreme cases pulmonary and cardiac arrest and death. Your child will be closely monitored and if an allergic reaction develops, he/she will be promptly treated with epinephrine and breathing assistance if necessary. Both therapies are immediately available in the room where the brain scan will be performed.

Patients at risk for injury from MR are those with pacemakers,

aneurysm clips (metal clips on the wall of a large artery), or shrapnel fragments.

Risk of Surgery:

The surgical risks depend on the preoperative condition of the patient, the nature of the operation, and the location and size of the tumor. Known risk associated with brain surgery include: hemorrhage, deterioration of neurological functions (such as weakness in the arm and/or leg, loss of sensation over parts of the body, and partial or complete loss of functions related to communication such as speech and comprehension, and other functions related to intellectual capacity, memory, etc.), infection and death. The relative risk will be discussed with you in accordance with your child's condition, specific findings and planned surgical procedures.

Risk of the Ommaya reservoir:

The Ommaya reservoir is a commonly used device in neurosurgery. The Ommaya reservoir is inserted under the skin with an attached tube that extends into the area of tumor. To use the Ommaya reservoir, the overlying skin is cleansed and needle is inserted through the skin into the chamber. The potential complications relating to the use of the Ommaya reservoir are infection and bleeding. Every effort will be made to maintain sterility and prevent bleeding.

Risk of the Vector/TK Gene Transfer:

Even though the vector-producing cells cannot grow and are considered harmless in humans, it is possible that events could occur within the cells that would permit the vector to grow and/or make the cells cancerous. Gene transfer using similar vectors has been used in adult and child patients. Since these experiments started in 1989, none of the more than 20 people who have received cells into which genes had been transferred by vectors has developed any problems related to the gene transfer method. We believe these vectors are safe and are not a threat to other people or to society.

This method of treatment has 2 major potential problems. First, the vector may be passed into surrounding normal tissue in addition to tumor tissue. We have not found any evidence of problems in mice and rats due to the spread of vector to surrounding normal brain tissue or to other sites in the body. We believe that some of the surrounding blood vessel cells probably do have the vector, but the number is too small to result in significant adverse side effects. It is possible that bleeding and neurologic symptoms (headache, convulsions, stroke) may develop with Cytovene.

Second, the vector-producing cells might persist in your child's body and cause cancer or other disease. We expect the immune system to reject (kill) the vector-producing cells in 1-2 weeks. Thus, they should not be able to survive and grow in your child's body. In addition, we expect the Cytovene therapy will kill all cells with the vector, including the vector-producing cells. Therefore, the vector-producing cells should not survive and the insertion of the vector should not result in new cancerous cells, since we think all of the cells with the vector will be killed by Cytovene. Therefore, we feel

that the risk of developing a new cancer is very small.
Risk of Cytovene Therapy:

Cytovene has been used extensively in humans to treat a number of infections including viral infections of the eye. We will be following the recommended dose for therapy that has been used to treat viral infections of the eye. The most commonly observed complication in people receiving Cytovene has been the development of decreased white blood cell and platelet counts. This could result in an increased risk of infection and bleeding. We will monitor these counts every other day (during Cytovene treatment) and the drug will be discontinued if the white blood count or platelet count drops significantly. Discontinuation of the drug results in normalization of the white blood cell and platelet numbers. Cytovene may cause permanent or temporary infertility and may be associated with birth defects. Therefore, women of childbearing age should use effective contraception during Cytovene therapy and men should use contraception during and for at least 90 days following Cytovene therapy. Pregnant women are not eligible for this protocol. Cytovene can cause cancer in animals. There is no information available to estimate the risk of this in humans.

Additional Risk:

This procedure is relatively new and it is possible that despite our extensive efforts other unforeseen problems may occur including the possibility of unknown and possible disabling effects or death.

A TENTATIVE AGREEMENT TO AN AUTOPSY

In case of death during or after your participation in this research study, we ask you for a tentative agreement to an autopsy. This will provide us with an opportunity to learn more about the effect of our treatment of the brain tumor.

ALTERNATIVE TREATMENTS

Your child has been offered the opportunity to participate in this study after your child's tumor recurred despite appropriate standard therapies for your child's disease. There are no other standard treatments that were shown to have significant effects in patients with your child's disease. A variety of experimental studies for the treatment of brain tumors are conducted in medical centers around the world, but the benefit of their approaches is as yet unknown. In addition, there is always the possibility for your child to decline any further treatment for his/her disease.

OTHER PERTINENT INFORMATION

1. When results of this study are reported at scientific and medical meetings or in journals, no patient will be specifically identified. Your child's medical records at Childrens Hospital Los Angeles will be maintained in accordance with legal requirements and will be made available only as required by approved members of the Food and Drug administration, National Cancer institute and

Genetic Therapy Inc. unless your written approval has been obtained.

2. This clinical procedure has attracted a great deal of attention from the media. We will make every effort to protect the confidentiality of your child and your family. However, because of this media interest there is a greater risk than usual that information concerning your child's treatment will appear publicly without your consent.

3. You are free to withdraw your consent for your child to participate in this study and seek care from any physician at any time. This will involve no loss of benefits and will not jeopardize your child's care in any way.

4. There is no financial compensation for participation in this study. Payment for the costs of your child's participation in this study will be sought from entities involved in this research study or your third party payer(s).

5. For answers to questions or should any problem arise regarding this research protocol, you should contact one of the following investigators.

   Corey Raffel, M.D., Ph.D. (Co-Principal Investigator)
   Stuart Siegel, M.D. (Associate Investigator)

6. We suggest you retain a copy of this document for future reference and personal records.

PHYSICAL INJURY

The possible risks associated with this study plan have been presented. There is always the possibility that physical injury may occur. The Childrens Hospital of Los Angeles maintains professional liability insurance to protect patients from financial losses resulting from physical injury caused through the fault of the hospital, its employees or its agents. If physical injury occurs in the absence of such fault, the hospital does not provide reimbursement for treatment expenses or other compensation for physical injury suffered as a result of your child's participation in this study.

If you believe that physical injury has been suffered as a result of participation in this study, you should contact Dr. Corey Raffel at (213) 669-2169, or Dr. Stuart Siegel at (213) 669-2205. Treatment for such injury will be available under the same financial arrangement as treatment is usually provided.

CONFIDENTIALITY

Case histories, the results of treatment, laboratory and pathologic data, photographs and X-rays may be published for scientific purposes, but neither your child's nor your identity will be disclosed and

confidentiality will be maintained. The Food and Drug Administration (FDA), the Department of Health and Human Services (DHHS) and/or their designate(s), and representatives from Genetic Therapy, Inc. may inspect the records relating to your child's participation in the study. If the study plan or use of the information is to be changed, you will be so informed.

VOLUNTARY PARTICIPATION

This study program has been reviewed, and its consent form has been approved by the Institutional Biosafety Committee (IBC), Committee on Clinical Investigations (IRB-Institutional Review Board), the Recombinant Advisory Committee (RAC) at the National Institutes of Health, and the Food and Drug Administration (FDA). The approval of these Committees does not alter the fact that the final decision to enter your child in the study program is yours. You have the right to remove your child from the study at any time. Dr. Corey Raffel and Dr. Stuart Siegel will answer any questions you may have regarding the study. Subsequent medical care of your child at the Childrens Hospital of Los Angeles is in no way contingent upon participation in this study program.

HOW TO OBTAIN INFORMATION

Daytime Monday through Friday, 8 a.m. to 5 p.m., you may call (213) 669-2169. You may leave a message with a secretary, and a doctor will return your call.

Evening, nighttime, and weekends or holidays: You may call the hospital number (213) 660-2450, and ask for the on-call doctor for the Neurosurgery Department. You may be asked to leave a message with the page operator, and the physician on-call will return your call as soon as possible.

If your questions are not an emergency, you can obtain better information by calling Dr. Corey Raffel, or Dr. Stuart Siegel, Monday through Friday, 8:00 a.m. through 4:30 p.m., than by calling the Neurosurgery Department doctor on-call after hours.

If you have questions regarding your rights or your child's rights as a patient/subject with regard to this study program, you can contact Ms. Gwenn Oki, Administrator of the Committee on Clinical Investigations (IRB) at (213) 669-2265.

SIGNATURE

Your signature below indicates that you have read this document, understand its meaning; have had a chance to ask questions; have these questions answered to your satisfaction; and consent to your child's participation in this study program.

You hereby give your consent for your child to be treated according to this clinical study program. You will be given signed copies of the Experimental Subject's Bill of Rights and this consent form.

Signature of Mother/Father (Date Signed)	Signature of Patient (Date Signed)
Signature of Investigator (Date Signed)	Signature of Witness (Date Signed)

CHILDRENS HOSPITAL LOS ANGELES
EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

Any person who is requested to consent to participate as a subject in a research study involving a medical experiment, or who is requested to consent on behalf of another, has the right to:

1. Be informed of the nature and purpose of the experiment;

2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized;

3. Be given a description of any attendant discomforts and risks reasonably to be expected from your/your child's participation in the experiment;

4. Be given an explanation of any benefits reasonably to be expected from your/your child's participation in the experiment;

5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to you/your child, and their relative risks and benefits;

6. Be informed of the avenues of medical treatment, if any, available to you/your child after the experimental procedure if complications should arise;

7. Be given an opportunity to ask any questions concerning the medical experiment or the procedures involved;

8. Be instructed that consent to participate in the experimental procedure may be withdrawn at any time and that you may discontinue participation in the medical experiment without prejudice;

9. Be given a copy of this form and the signed and dated written consent form.

10. Be given the opportunity to decide to consent or not to consent to the medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on your decision.

You have carefully read the information contained above in the "Experimental Subject's Bill of Rights" and you understand fully the rights of a potential subject in a medical experiment involving people as subjects.

_____ _____
Date Parents/Legal Guardian

_____ _____
Time Patient

ESBR.doc
6/92

_____
Witness

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

CVs

NAME	POSITION TITLE
Corey Raffel, M.D., Ph.D.	

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Harvard University, Cambridge	A.B.	1974	
University of California, San Diego	M.D.	1980	
University of California, San Diego	Ph.D.	1980	
University of California, San Francisco	Internship	6/80 - 6/81	General Surgery
University of California, San Francisco	Residency	7/81 - 7/86	Neurosurgery

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL BACKGROUND

Clinical Instructor, Neurological Surgery
University of California, San Francisco
1/87-6/87

Assistant Professor of
Neurological Surgery
University of Southern California
School of Medicine
7/87 - 6/30/92

Associate Professor of Neurological
Surgery
University of Southern California
School of Medicine
7/1/92 - present

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Raffel C, Gilles FE, Weinberg KI. Reduction to Homozygosity and Gene Amplification in Central Nervous System Primitive Neuroectodermal Tumors of Childhood. Cancer Res. 50:587-591, 1990

Raffel C, Litofsky NS, McComb JG. Central Nervous System Malformations and the VATER Association. Ped Neurosurg 16:170-173, 1990-91.

Thomas GA, Raffel C. Loss of Heterozygosity on 6q, 16q, and 17p in Human Central Nervous System Primitive Neuroectodermal Tumors. Cancer Res. 51:639-643, 1991.

EDUCATION (CONTINUED):

Internship:	1980 University of California, San Francisco General Surgery 6/80-6/81
Residency:	University of California, San Francisco Neurological Surgery 7/81-7/86
Fellowships:	Hospital for Sick Children, Toronto Pediatric Neurosurgery 7/86-12/86
	University of California, San Francisco Pediatric Neurosurgery 1/87-6/87

BIBLIOGRAPHY (CONTINUED):

Raffel C, Neave VCD, Lavine S, McComb JG. Treatment of Spinal Cord Compression by Epidural Malignancy in Childhood. Neurosurgery 28:349-352, 1991.

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Karnes PS, Tran TN, Guy MY, Raffel C, Gilles FH, Barranger JA, Ying KL. Cytogenetic Analysis of 39 Pediatric CNS Tumors. Cancer Genetics & Cytogenetics 59:12-19, 1992.

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Rabb CH, McComb JG, Raffel C, Kennedy JG. Spinal Arachoid Cysts in the Pediatric Age Group: An Association with Neural Tube Defects. J Neurosurg. In press.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Kenneth K. Culver, M.D.	Clinical Director

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Drake University, Des Moines, Ia	B.A.	1973-77	Biology
University of Iowa, Iowa City, Ia	M.D.	1977-81	Medicine
University of California, San Francisco		1981-85	Pediatrics
University of California, San Francisco		1985-87	Fellow-Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the pro Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the maste baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membershi any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

National Institutes of Health, Bethesda, Md	1987-90	Clinical Associate
National Institutes of Health, Bethesda, Md	1990-92	Sr Clin. Investigator
Iowa State University, Ames Iowa	1992-	Professor-Genetics
Iowa Methodist Medical Center, Des Moines, Ia	1993-	Director-Res. Institute

Diplomat, American Board of Pediatrics (1986)
Diplomat, American Board of Allergy and Immunology (1989)

Honors and Awards

1977	Omicron Delta Kappa Honor Society
1977	Beta Beta Beta Biological Honor Society (President)
1981	Sandoz Award for outstanding clinical and research activities in Child Psychiatry
1986	Bank of America-Giannini Foundation Medical Research Fellowship
1990	Drake University Young Alumni Award
1991	BioEast '90 Prize for Outstanding Technical Innovation -- The production of high titer retroviral vector preparations using a hollow fiber cell culture system
1992	Harvard Health Letter - "First human gene therapy, the most important advance in medical research in 1991"

Membership in Professional / Scientific Societies

American Association of Immunologists
Clinical Immunology Society
American Association for the Advancement of Science
American Academy of Pediatrics

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Date of RAC Approval: 6/8/1993
Date of NIH Approval: 9/3/1993

A ONE-PAGE SCIENTIFIC ABSTRACT OF THE PROTOCOL:

We propose a protocol to transduce human bone marrow cells with the multiple drug resistance (MDR) gene. This is done to increase the expression of MDR in human bone marrow cells that usually express very low levels of this important glycoprotein. High MDR-producing marrow cells are necessary to prevent killing of these cells with several classes of chemotherapeutic agents, including taxol, anthracyclines, vinca-alkaloids, and etoposides. Usually, when high doses of chemotherapeutic agents including those mentioned above are employed, significant bone marrow toxicity is induced. To avoid this toxicity, autologous bone marrow transplantation (ABMT) (the removal of human bone marrow and its reinfusion) is done in association with the administration of high-dose chemotherapy for patients with advanced cancer.

The transduction of the removed bone marrow cells by a retrovirus containing the MDR gene is proposed. These transduced cells will be reinfused into patients as is ordinarily done in ABMT with high-dose chemotherapy. It is hoped that the expression of MDR in the transduced bone marrow cells will induce resistance of these cells to the toxic effects of subsequent chemotherapy. A safe and efficient line of cells producing a retrovirus efficient in transferring the MDR gene into animal bone marrow cells will be used in this study and is expected to have no toxicity. The potential benefit of the protocol is that it may create a population of human bone marrow cells resistant to the toxicity of high-dose chemotherapy with drugs such as taxol, an MDR-responsive agent in patients with advanced breast and ovarian cancers, and either allow these patients to receive higher doses of taxol or allow patients to be treated with taxol who could not tolerate this therapy because of its bone marrow toxicity. If this treatment is successful, it could be used to administer higher dose chemotherapy to patients undergoing ABMT at earlier times and increase the possibility of prolonged survival or cure in these patients.

A ONE-PAGE DESCRIPTION OF THE PROPOSED EXPERIMENT IN NON-TECHNICAL LANGUAGE:

In the treatment of patients with metastatic cancer, i.e., cancer that has spread beyond the primary site involved, the administration of several anti-cancer drugs is usually required. High doses of these drugs is so damaging to normal human bone marrow cells that removal of some bone marrow from the patient prior to being given the drug is routinely performed; this marrow is then put back into the patient after the effects of the chemotherapy are gone.

The goal of these studies is to transfer a gene into the patient's normal bone marrow cells when they are removed (bone marrow transplantation) as ordinarily occurs in treatment to make these cells resistant to the effects of these drugs. The result of this procedure will be the creation of normal bone marrow cells more resistant to some types of routinely used anticancer drugs. Bone marrow expressing this new added gene should be resistant to the killing effect of these drugs. The patient can, therefore, produce more normal levels of red and white blood cells and platelets during chemotherapy. This may confer a significant advantage to patients with cancer that has spread, especially when given subsequent rounds of chemotherapy. Theoretically, the more chemotherapy the patient receives after getting the drug-resistant gene the more resistant bone marrow cells will remain in the patient since bone marrow cells that do not express the added gene will be preferentially killed. This treatment, if successful, could allow patients with advanced cancer to receive high doses of potentially effective drugs, or allow patients to be treated with drugs who could not otherwise be treated because of the side effect to their bone marrow.

The potential harm of the use of this "gene therapy" is minimal. Animal studies and other laboratory tests have shown this type of gene therapy to be safe. Thus, this gene therapy treatment may benefit patients with cancer that has spread but not involving the bone marrow. We are trying this therapy first in patients with advanced breast cancer in the hope that we can decrease the damage done by drug treatment to the bone marrow, and allow more and higher doses of drugs to be given to these patients. At future times, this treatment, if successful, could lead to higher doses of chemotherapy to be given earlier, and a greater chance of remission and possible cure.

PROPOSAL FOR HUMAN GENE THERAPY SUBMITTED TO THE RECOMBINANT DNA ADVISORY COMMITTEE (RAC) OF THE NIH

HUMAN MDR GENE TRANSFER IN PATIENTS WITH ADVANCED CANCER

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1. BACKGROUND MATERIAL:

A. SUMMARY:

Intensive cancer chemotherapy usually requires autologous bone marrow transplantation (ABMT) because of the dose-limiting toxicity to bone marrow cells of the chemotherapy used. This approach is especially useful for tumors not involving the bone marrow. In many cases, the toxicity is due to the low levels of expression of the human multiple drug resistance (MDR1) gene in human bone marrow cells. The goal of this proposal is to transfer and express the human MDRI (MDR) gene in human bone marrow cells of patients with solid tumors not involving the bone marrow who undergo ABMT in association with high-dose chemotherapy; this procedure should protect the marrow from bone marrow toxicity on subsequent administration of MDR-responsive anti-cancer drugs (anthracyclines, vinca alkaloids, etoposides, and taxol). We have demonstrated efficient and stable transfer of the human MDR gene into mouse bone marrow stem cells, and a concomitant marked increase in expression of MDR in these cells. In addition, we have shown that enrichment of the population of MDR-expressing cells occurs on treatment of these live mice with taxol. We have also shown that human bone marrow precursor ((CD34+) cells can be transduced with the human MDR gene and increase their expression of MDR after gene transfer. All these studies have been performed with supernatants from a safe and efficient MDR retroviral producer line developed in our laboratory.

In this proposal, we present a protocol for the use of human MDR gene transfer into the bone marrow cells of patients undergoing ABMT in association with chemotherapy for advanced cancers. This protocol has two goals: 1) To analyze the extent of transfer and expression of the human MDR gene into the bone marrow cells of humans; and 2) To determine the toxicity of this treatment. In patients on this protocol who are subsequently treated with taxol, an MDR-responsive drug, we will also obtain information about whether increased MDR expression leads to enrichment for MDR-transduced bone marrow cells. This induction of high MDR expression, if successful, may lead to decreased toxicity to the bone marrow when patients are subsequently treated with MDR-responsive drugs. If successful, this strategy may also permit significant increases in the dose intensity of drugs useful in advanced cancer such as taxol and adriamycin, and lead to longer survival of patients and the possibility of cure.

2. SPECIFIC AIMS:

Intensive cancer chemotherapy usually requires concomitant autologous bone marrow transplantation (ABMT) because of the dose-limiting bone marrow toxicity of the chemotherapy used. This approach is especially useful for tumors not involving the bone marrow. In many cases, this marrow toxicity is due to the inadequate level of expression of the multiple drug resistance MDR1 (MDR) gene in normal bone marrow cells; MDR protein pumps certain drugs and other substances out of cells. The goal of this proposal is to use safe, defective retroviruses to transfer and express the MDR gene in the bone marrow cells of patients with advanced cancer undergoing ABMT who may subsequently be treated with drugs that require MDR for their inactivation (MDR-responsive drugs) commonly used in cancer chemotherapy; these include anthracyclines, vinca alkaloids, etoposides, and taxol. This strategy, if successful, should enrich the marrow for transduced MDR-expressing cells, and reduce bone marrow toxicity associated with subsequent exposure to these drugs. These studies may also result in safer and more feasible higher dose chemotherapy for patients with advanced cancer which may lead to longer survival and increased chances of cure for these patients.

The specific aims of this protocol are: 1) To analyze the extent of transfer and expression of the MDR gene into the bone marrow cells of humans with advanced cancers; and
2) To determine the toxicity of this treatment. In patients subsequently treated with taxol, an MDR-responsive drug, we will have the opportunity to evaluate if this treatment leads to enrichment for MDR-transduced and expressing bone marrow cells. This may lead to decreased marrow toxicity following taxol treatment.

3. BACKGROUND DATA AND SIGNIFICANCE:

Successful HLA-compatible allogeneic bone marrow transplantation in humans is a major impetus for these studies (1-4). The availability and therapeutic advantage of autologous bone marrow transplantation (ABMT) (5-8) provides additional enthusiasm for this approach. The goal of the studies in this grant is to provide a new approach to avoid bone marrow toxicity in patients with cancer. To accomplish this, we plan to use the human MDR1 (MDR) gene to select bone marrow cells that contain and express this gene. Normal human bone marrow cells contain low levels of MDR, and, thus, are particularly susceptible to the toxicity induced by certain classes of drugs (MDR-responsive drugs). The general scheme to accomplish this gene therapy and overcome the toxicity will be: 1) Removal of bone marrow cells

from patients routinely undergoing ABMT in association with high-dose anti-tumor chemotherapy for solid tumors not involving the bone marrow; 2) Insertion of the human MDR gene into these cells using retroviral vectors; 3) Treatment of the patient with high-dose chemotherapy; and 4) Reinfusion of the transduced bone marrow cells containing and expressing the transferred genes. On subsequent exposure to MDR-responsive drugs, the marrow of these patients should be enriched for cells resistant to the chemotherapy; the reduced marrow toxicity may permit escalation of doses of chemotherapy in the hope of cancer cure in selected patients.

Efficient and safe gene transfer and high-level MDR expression are the major goals in this gene therapy-autotransplantation scheme. We will use: 1) Retroviral vectors that maximize MDR gene transfer and expression into human bone marrow cells; 2) Purification of bone marrow hematopoietic precursor cells (BM-HPC) using antibodies and cell-sorting to increase the ratio of virus:cells; 3) Growth factors to increase cycling necessary for retroviral infection and the number of BM-HPC; and 4) *In vitro* assays to assess the number of BM-HPC, and assays for human MDR DNA and MDR protein to determine MDR transfer and expression, respectively.

The background for the proposed protocol includes progress made recently in the following areas:

A. Transfer and Expression of Foreign Genes into Mice:

Retroviral vectors are the most efficient means of gene transfer described to date (9). Defective packaging lines having mutations that do not allow the encapsulation of an intact Moloney murine leukemia virus genome are available (10-17). These packaging lines transfected with retroviral vectors are called producer lines. Retroviral vectors have been used to transfer the neomycin resistance (neo^R) gene into mouse bone marrow stem cells; this results in reconstitution of the host mainly with cells that contain the transferred gene (18-24); this is especially true if preselection of cells with G418, a neomycin analogue, is used *ex vivo* (21,23,24). This is important because if donor stem cells are untransduced they will compete with transduced stem cells in reconstituting the host marrow. From these experiments, we infer that a selectable gene transferred along with a co-transferred non-selectable gene on the same retroviral vector would enrich for cells containing both genes. G418 cannot be used in live animals because of its toxicity, and, thus, the MDR gene has been chosen as a selectable gene. Human ADA (25), CD8 (26), ß globin (27-29), and glucocerebrosidase (30)

genes, as well as the human MDR gene (31,32), have all been successfully and stably transferred into the bone marrow cells of live mice, and, in most cases, expressed.

B. The Use of the MDR Gene as a Selectable Marker:

The human MDR gene, when expressed in cells, provides resistance to a variety of commonly used anti-cancer compounds including the anthracyclines, vinca alkaloids, etoposides, and taxol (33,34). (These and other drugs susceptible to MDR activity are referred to as "MDR-responsive" agents or drugs.) The cDNA for the human MDR gene has been cloned and is expressed in both mouse and human cells in culture (35,36). Retroviruses containing the MDR gene are also available (37,38). The expression of this gene leads to resistance of cells in culture to mitomycin and colchicine (34,38). The MDR gene has been transferred into mouse and human bone marrow cells in culture and leads to drug resistance of the transduced cells (39). A transgenic mouse line expressing the MDR gene only in the bone marrow is resistant to the effect of daunomycin, a drug that decreases the white blood cell count of normal mice (40,41). Inhibitors of MDR gene expression are available that lower the level of MDR and allow increased cell killing with MDR-responsive drugs (34,41,42). Specific oligonucleotide primers can be used to detect the MDR gene and its RNA product in cells by polymerase chain reaction (PCR) analysis (43). Expression of the human MDR gene at the protein level can be detected by antibodies (44-46). The MDR gene has recently been shown to be stably transferred and expressed in both MELC (47) and the bone marrow cells of live mice (31,32).

C. Methods to Increase Infection and Expression of Transferred Genes:

Repeated administration of retrovirus containing an ADA gene in long-term bone marrow culture increases the efficiency of gene transfer (48). Stromal cell lines support the growth of bone marrow cells optimally in short- and long-term cultures (49,50). Certain growth factors alone and in combination, primarily IL-3, IL-6, GM-CSF, and stem cell factor (SCF), also known as mast cell factor and c-Kit ligand, enhance retroviral gene transfer into murine bone marrow stem cells (51-61). These factors increase the cycling and proliferation of bone marrow stem cells (56-62).

Monoclonal antibodies have been used to enrich human bone marrow cells for early hematopoietic cells containing the majority of bone marrow stem cells (BM-SC) (63-67). These BM-SC are defined here as cells capable of long-term reconstitution of all

hematopoietic cell lineages (red cells, granulocytes, platelets, macrophages, and lymphocytes), and are also a self-renewing population. Anti-CD34 has been used to enrich for BM-SC and is capable of reconstituting ablated human marrows (64). In these recent experiments, a CellPro Corporation CEPRATE™ Stem Cell Concentrator has been used (64,65). This system provides a closed system in which bone marrow cells are passed through a column of beads containing biotinylated anti-CD34 antibody 12.8 (65). Several other antibodies including anti-CD33, anti-CD38, anti-DR, and anti-Thy1 have also been used (66-69). This enrichment for hematopoietic precursor cells containing BM-SC is desirable and necessary because it concentrates the BM-SC into a small volume facilitating optimal transduction with supernatants of MDR retroviral producer cells and incubation with growth factors. These conditions also: 1) Favor BM-SC replication required for retroviral gene transfer; 2) Increase the virus:target cell ratio; and 3) May increase the number of stem cells by replication.

Lethally irradiated mice given both mouse and human bone marrows depleted of B and T lymphocytes survive, and support the growth of human B and T cells for 6-8 months (70). Immune-deficient (SCID) mice also support the survival and growth of human bone marrow cells (71-75). The most successful model to date has been SCID mice given PIXY321, a fusion protein of IL-3 and GM-CSF in combination with SCF and GM-CSF (74). In these mice: 1) Large numbers of human myeloid and erythroid precursors are present in marrow over 1-2 months; 2) Human marrow stem cells or very early hematopoietic precursors are present; and 3) Repopulation with human marrow is dependent on PIXY321.

Transfer of neo^R (76,77) and adenosine deaminase (ADA) genes (78) into the lymphocytes of human subjects have recently been reported. These experiments have been approved in humans by the RAC and the FDA. ABMT in humans is routinely used in association with intensive cancer chemotherapy to prevent life threatening bone marrow toxicity (5-8). The high-level expression of the MDR gene, normally expressed at very low levels in bone marrow, should provide resistance of treated marrow to the toxic effects of subsequent chemotherapy with MDR-responsive drugs (79).

Another approach to gene therapy is by correcting a gene defect in the involved cells by homologous recombination (80-82). In the case of MDR, this procedure could conceivably be used to add a promoter or enhancer to the normal relatively inactive MDR gene in bone marrow cells. However, in experiments to date, the frequency of appropriate homologous recombination leading to gene correction is $1/10^6$-10^7 treated cells (80-82), and it is not yet possible to select and grow rare hematopoietic cells with the

correct recombinational event in sufficient quantity. This is the major reason we favor the use of retroviruses to add a normal MDR gene with a strong promoter as the current method of choice to pursue the goal of gene therapy. Adenovirus and adeno-associated vectors (AAV) have also been used recently to transfer genes into cells (83,84).

4. RELEVANT PRELIMINARY STUDIES FROM OUR LABORATORY:

A. Transfer and Expression of Foreign Genes in Mice:

We have developed unique retroviral ecotropic and amphotropic packaging lines for safe and optimal gene transfer (16,17). These packaging lines contain the gag-pol and env genes on different plasmids to avoid recombination. The packaging cell lines that produce the highest titer ecotropic and amphotropic viruses are GP+E-86 and GP+envAM-12, respectively (16,17). These lines are both safe and highly efficient in gene transfer, and have been sent to >300 laboratories worldwide with no reports of wild-type virus production. Recently, GP+envAM-12 was approved by the RAC and the FDA for use in cancer patients with an IL-2 gene-containing retroviral vector (85).

We have used the GP+E-86 packaging line to transfer the neo^R gene in a retroviral vector (N2) into irradiated mice (24). In these studies, we demonstrated high-level gene transfer; ~50% of the mice show neo^R gene integration in spleen colonies 13 days posttransplantation by Southern blotting (24). These spleen colonies are presumed to represent proliferation of individual early bone marrow cells in the mouse and may be a reflection of true multipotent bone marrow stem cells (86). Transplantation of spleen colonies from neo^R-transduced mice into secondary-irradiated recipients supports this conclusion (24). Mouse bone marrow at later times also shows neo^R gene integration. The number of sites of integration detected at late times is 1-5 (24), consistent with previous reports suggesting that only a few stem cells are involved in repopulation (21,23,24,87,88). In addition, most or all of the hematopoietic cells in both spleen colonies and bone marrow contain the neo^R gene as assessed by the intensity of the neo^R signal on Southern blots as compared to a single copy (globin) gene (24). Thus, a foreign gene can be stably transduced into the majority of bone marrow progeny in live mice.

B. The MDR Gene as a Selectable Marker:

We have shown that the human MDR gene can be successfully and stably transduced and expressed in mouse erythroleukemia cells (MELC) (47) and mouse bone marrow cells

(32). In these studies, we have collaborated with Drs. Ira Pastan and Michael Gottesman (National Cancer Institute) in the use of an MDR cDNA in a retroviral vector constructed by these investigators (37) (Figure 1). This retroviral vector contains the entire coding sequence of the MDR gene and its expression driven by Harvey leukemia virus LTRs, and also contains a Harvey LTR enhancer. We have transfected this retroviral vector into our ecotropic and amphotropic packaging lines, and selected producer cells containing and expressing the MDR gene by exposure to colchicine (32,39). Cells that do not express the MDR gene are killed, while cells producing high levels of MDR survive. We have isolated several surviving clones and chosen those with the highest viral titers: 5×10^5 viral particles/ml for the ecotropic line, and 5×10^4 viral particles/ml for the amphotropic MDR producer line. We have used several transfect/infect protocols in an attempt to increase retroviral titers, but without success. In this scheme, we transfected the MDR-containing retroviral vector into our GP+E-86 ecotropic packaging cells, and selected for colchicine-resistant clones. We then used viral supernatants from the highest titer ecotropic clone and infected amphotropic packaging cells. Clones were selected for colchicine resistance and titered on uninfected 3T3 cells.

We cocultured our highest titer (5×10^5 particles/ml) ecotropic producer clone with mouse bone marrow cells (32), and transfused the transduced mouse bone marrow cells into lethally irradiated mice as described (32). We demonstrated the presence of the human MDR gene in the peripheral blood of 90% of the transplanted mice using PCR analysis with human MDR-specific oligonucleotide primers (43) 50 days posttransplantation (32). By contrast, no MDR signal is seen in the tail vein blood of untransplanted mice.

In other experiments, we have examined the long-term expression of the human MDR gene in mice, and the cell types in which the inserted gene is expressed. At eight months posttransplantation, 50% of the successfully transplanted mice continue to contain the human MDR gene by PCR of peripheral blood (32). Southern blots of bone marrow from sacrificed animals demonstrate human MDR (90).

To analyze the expression of the human MDR gene at the protein level, we have used a monoclonal antibody, 17F9 (46), reacting with an external epitope of MDR p-glygoprotein on the surface of cells (provided by Dr. David Ring of Chiron Corporation). Cells are first reacted with 17F9 and then exposed to a fluorescent anti-IgG2b (32). Using 17F9 in fluorescent-activated cell sorting (FACS) analysis, we have shown that clones of both producer 3T3 cells and MELC containing the MDR cDNA retroviral

vector have a 1-2 log increase in MDR activity (32,47). In addition, a significant number of bone marrow cells from live mice transduced with MDR virus express high levels of MDR eight months posttransplantation. To rule out the possibility that long-lived lymphocytes are the source of the MDR positive cells, cell gating on the basis of size and morphology was used to exclude lymphocytes from the analysis (32). Approximately 14% of the granulocyte-macrophage cells in the bone marrow of a mouse eight months posttransplantation contain markedly increased amounts of MDR p-glycoprotein on their surface as compared to controls (32). These data show that MDR-positive cells in this granulocyte-macrophage population are derived from bone marrow stem cells.

We also showed that we can select *in vivo* for MDR-transduced cells in bone marrow (32). Four transduced mice, initially positive by PCR for the MDR gene, subsequently lost their MDR PCR signal at eight months posttransplantation. These mice were given 140 μg of taxol in an attempt to enrich for bone marrow cells containing and expressing the human MDR gene, and to prove that MDR expression allows cell selection. Seven days after a single dose of taxol, the PCR signal in all four mice reappeared; in addition, FACS analysis of the peripheral blood of two of these mice showed 5-8% MDR-positive granulocytes (32).

We have also recently transferred the human MDR gene into live mice using MDR-containing cell-free supernatants from retroviral amphotropic producer cells. Thus, we have demonstrated that: 1) Bone marrow stem cells can be stably transduced with the human MDR gene for long periods of time; and 2) MDR-transduced cells are protected from taxol toxicity and can be selected. In these studies, PCR analysis is used to detect MDR DNA in tail vein blood and bone marrow (43). Southern blot analysis also measures MDR DNA (89,90), and Northern blotting and S1 nuclease analyses quantitate MDR RNA (89,91-93). In MELC transduced with MDR virus-containing producer cells, we show positive Southern and Northern blots using human MDR-specific probes (47). Thus, we have established conditions for the analysis of MDR DNA and mRNA by Southern and Northern blotting, and for the presence and quantitation of MDR protein by FACS analysis. We have also demonstrated high-level expression of a stably transduced human MDR gene in 3T3 cells, MELC, and the bone marrow cells of live mice both short- and long-term after transplantation with MDR-transduced cells (32,47).

Human bone marrow stem cells (or early precursor cells) have rhodamine123Low (Rho^{Low}) staining, and have high levels of MDR expression (94); this Rho^{Low}staining is probably due to the

ability of MDR-expressing cells to pump out Rhodamine dye (94). It was implied that this higher level of MDR gives a meaningful selective advantage to stem cells when challenged with MDR-responsive drugs, and that transduction with an exogenous MDR gene would have little or no additional effect. We and others have shown that this is not correct: 1) Normal mice can be made severely leukopenic after treatment with daunomycin, while transgenic mice, whose bone marrow contains an exogenous human MDR gene regulated by a strong promoter, are unaffected (40,41); 2) Cancer patients treated with MDR-responsive drugs have severe bone marrow toxicity (1-8); and 3) MDR-transduced bone marrow cells in live mice are preferentially protected from toxicity and enriched on exposure to taxol (31,32).

If untransduced marrow stem cells were normally protected by their relatively higher levels of MDR, there should be little if any marrow toxicity induced by chemotherapy. This is clearly not the case. In addition, if transduction and expression of an exogenous MDR gene was not significant, then selection of MDR-transduced cells should not be possible. The data support the hypothesis that: 1) Although bone marrow stem cells have a relatively increased amount of MDR gene expression, this increase is not great enough to prevent bone marrow toxicity in mouse or human marrow exposed to MDR-responsive drugs; and 2) Transduction and expression of MDR using an exogenous gene on a strong promoter results in resistance and selection of transduced murine marrow stem cells on exposure to MDR-responsive drugs, and, thus, is a rational approach to protecting human bone marrow cells from the toxicity of these drugs. This does not imply that MDR transduction is the only means of decreasing bone marrow toxicity in the treatment of patients with cancer undergoing chemotherapy with ABMT. Cytokines such as GM-CSF have been shown to shorten the time to marrow recovery (95,96); peripheral blood stem cell infusions are useful as well (97-100). Combinations of these therapies with MDR transduction may be complementary, and more beneficial than any used alone to decrease or eliminate marrow toxicity.

C. Human Marrow Transduction with the MDR Gene using a Safe Amphotropic MDR Producer Line:

In other experiments (101-107), we have transduced human bone marrow cells obtained from marrow harvests for future ABMT with our highest titer (5 X 10^4 particles/ml) amphotropic producer line, A12M1 retrovirus (Figure 2). Supernatants from A12M1 have been used instead of coculture with MDR producer cells to avoid potential contamination of the bone marrow with the producer cells, an undesirable side-effect in clinical use. In one set of experiments, we have cultured Ficoll-separated

nucleated bone marrow cells (NBMC) from whole marrow for 24 hours with media containing 10% fetal calf serum, 10 units/ml IL-3, 200 units/ml IL-6, and 50 units/ml human SCF (a gift from AMGEN) (107). The NBMC were then exposed to A12M1 supernatants for 4-8 hours, 3-4 X, over 48 hours to optimize retroviral transfer by providing an excess of virus over cells (usually 2-4 X 10^7 viral particles/10^7 cells) (107). PCR analysis with MDR primers from different exons yields a unique 157 basepair band from transduced integrated MDR cDNA; the endogenous human MDR gene containing introns gives either no signal or a band of larger size. A positive MDR PCR signal has been obtained after AM12M1 supernatants are exposed to human bone marrow cells (107) (Figure 3). In addition, we have shown that increasing the number of times the supernatant is changed increases the PCR signal until excess virus is added (107) (Figure 1). By analysis of BFU-E from transduced marrows, 10-50% of the colonies contain and express MDR as assayed by: 1) MDR PCR of individual colonies; and 2) Resistance of colonies to 60 ng/ml of colchicine, or both. In one experiment, 10% of BFU-E were MDR PCR-positive and colchicine-resistant (107). In two other experiments, six of 12 and five of 12 individual BFU-E were MDR PCR-positive after transduction. We have also demonstrated that 20-25% of human marrow cells transduced with MDR express the MDR protein at increased levels by FACS analysis (Figure 4). We have also used CD34+ cells isolated from CD34 antibody-coated plates (Applied Immune Sciences), and can show MDR transduction of these cells as well (107).

GP+<u>env</u>AM-12 packaging cells have been shown to be safe by their use with the IL-2 gene in human melanoma cells in culture (85), an ADA gene in monkey bone marrow transfer experiments (108), and in our own studies with MDR of A12M1 cells (32,47). Reverse transcriptase assays, Mus dunni co-culture studies, and utilization of supernatants on naive 3T3 cells, have all been used in these experiments. The GP+<u>env</u>AM-12 packaging cells have been approved by the RAC and the FDA for use with the Il-2 gene to treat patients with malignant melanoma (85).

We have established a collaboration with Dr. John Dick (University of Toronto), and currently are studying SCID mice infused with both untransduced and MDR-transduced human bone marrow cells using the protocol described earlier (107). In these experiments, 2-4 X 10^7 bone marrow cells have been given via tail vein to each mouse. This system should serve as a live animal model to determine the efficiency and potential toxicity of the transfer and expression of the MDR gene in human bone marrow cells prior to human trials.

TOXICITY:

We are aware of recent experiments in which three of seven monkeys whose bone marrow was transduced with high-titer retrovirus developed lymphoma shown to be due to insertion of wild-type amphotropic virus components (109). This toxicity is most likely due to the presence of intact amphotropic retrovirus in the retroviral stocks used. Assays for intact non-defective retroviruses using naive 3T3 or HeLa cells should have detected the presence of these dangerous retroviruses. As indicated earlier, our amphotropic GP+envAM-12 packaging line has been used extensively to generate retrovirus with retroviral vectors containing many different genes, and no evidence for intact retrovirus has been obtained from these producer lines, as assessed by assay on naive 3T3 cells. The use of GP+envAM-12 has been approved by the RAC and the FDA for human use (85).

We have used several different assays to test for intact retroviruses in the supernatants of our MDR amphotropic producer cells (A12M1) to be used in the proposed protocol. A 3T3 amplification assay with A12M1 supernatants has consistently been negative by reverse transcriptase. The supernatants of transduced human bone marrow cells is also negative. In addition, we are using the S+L assay and Mus dunni cells to assay for virus as well (110). To date, all our studies have been negative.

5. THE PROTOCOL:

A. Human Cancer Results:

The goal of this protocol is to evaluate the transfer and expression of the MDR gene in the bone marrow cells of human subjects with advanced cancer. These studies require approval of the RAC and the FDA as well as our Institutional Review Board (IRB).

The first of these clinical protocols will involve the transfer of A12M1 supernatants into the bone marrow cells of patients with advanced cancer undergoing ABMT as part of protocols whose ultimate goal is to reverse the bone marrow toxicity of chemotherapy. ABMT accompanying high-dose anti-cancer chemotherapy is currently being used in these patients who have no bone marrow involvement at Columbia-Presbyterian Medical Center (CPMC) as well as elsewhere. After MDR transduction, some of these patients may be treated with MDR-responsive drugs, such as taxol (and adriamycin).

These cancer patients are selected because: 1) They often

undergo ABMT during the course of their treatment; 2) They are not usually cured by current therapy; and 3) Taxol and adriamycin are MDR-responsive drugs often given to these patients following relapse with other agents.

Patients with metastatic cancer will be studied in our first protocol. Breast cancer patients with metastatic disease are initially treated with cyclophosphamide, 5-FU, and either methotrexate or adriamycin. They often relapse, and are then treated with high-dose chemotherapy such as platinum, cyclophosphamide, and thiotepa, but require ABMT to allow this regimen to be tolerated. We propose to transduce the bone marrow of these patients with MDR at this time. We will measure MDR gene transduction by PCR analysis and Southern blotting and MDR expression by measuring MDR mRNA and protein. Potential toxic effects will also be evaluated. We will also evaluate the potential marrow-protective effect of MDR gene transduction in patients who subsequently receive taxol. If the MDR gene is protective, bone marrow toxicity as reflected by decreased WBC and platelet counts and decreased bone marrow cellularity should be less severe than in patients not transduced with the MDR gene.

Our protocol in patients with advanced cancer has two primary goals. In all patients undergoing ABMT and MDR gene transduction, we will evaluate: 1) The transfer and expression of the MDR gene and its stability in bone marrow cells over time (see below); and 2) Any toxicity from the transduction procedure. We will also have the opportunity to test the effect of MDR transduction on marrow toxicity in those patients subsequently treated with taxol in enriching the marrow for MDR-transduced cells and in decreasing marrow toxicity.

Patients with advanced ovarian cancer undergoing ABMT and high-dose chemotherapy will also be eligible for this protocol. These patients are less likely to have marrow tumor infiltration. In addition, taxol has been shown to be an effective agent in the treatment of these patients.

Patients with high-grade brain tumors undergoing ABMT and high-dose chemotherapy will also be eligible to enter this protocol.

It should be noted that this initial protocol is designed primarily to assess the transfer and expression of the MDR gene and its safety. However, patients with advanced breast and ovarian cancer have a strong possibility of being treated with MDR-responsive agents such as taxol following marrow transduction. Treated patients should provide information on whether the added MDR gene can prevent or decrease bone marrow toxicity by enriching for cells resistant to these drugs. If MDR

gene transduction is shown to be safe, the results could then be used to design a subsequent trial whose goal would be to establish the efficacy of this treatment by either: 1) Changing the transduction protocol; or 2) Escalating doses of the MDR-responsive drugs used, i.e., taxol or adriamycin, following marrow transduction, to obtain an improved therapeutic result, i.e., prolonged survival of these cancer patients.

These studies will be carried out initially at CPMC, Sloan-Kettering Cancer Center, and Montefiore Hospital. Dr. Charles Hesdorffer, a participant in the mouse transplantation experiments and Director of the Bone Marrow Transplantation Program at Columbia University, and Dr. Karen Antman, Director of the Division of Oncology at Columbia University beginning July 1, 1993 and an investigator with experience in ABMT, will be co-principal investigators. Drs. Peter Wiernik (Montefiore) and Subhash Gulati (Sloan-Kettering) will also collaborate (letters attached). There are more than adequate numbers of patients available at these centers to carry out this protocol. The details of the trial are in the protocol at the end of this section.

Patients will be hospitalized for 24 hours to undergo the bone marrow harvest as is standard practice at CPMC. Greater than 3×10^8 cells/kg will be harvested from each individual who agrees to undergo the gene transfer procedure. The marrow will first be treated to separate mononuclear cells with a Ficoll-Hypaque density gradient. Once this is completed, 30-50% of the cells will be sorted for CD34+ cells depending on the number of cells harvested. We will use both CellPro columns (Ceprate™SC Stem Cell concentrator) containing beads with biotinylated anti-CD34 antibody, 12.8 (64,65), and AIS CD34 antibody-coated plates with anti-CD34 antibody, ICH3 (112), to purify CD34+ cells prior to transduction. An aliquot of the CD34+ population of cells will be plated in methylcellulose for CFU-GEMM and BFU-E assays directly. The bulk of the CD34+ marrow cells will be pre-incubated for 24 hours with growth factors (IL-3, IL-6, and human SCF), and then co-cultured with MDR virus producer cell AM12MDR supernatants; an aliquot will be taken for CFU-GEMM and BFU-E analyses. The cells will be kept in culture for 3-4 days during which time the patients will be given high-dose chemotherapy and will be prepared to receive MDR-transduced autologous bone marrow cells. At this time, the patients will receive enough nucleated cells of their own untreated unfractionated stored marrow to permit marrow reconstitution alone, in addition to the transduced marrow, representing approximately the same number of total marrow cells. In this way, we will ensure marrow engraftment and avoid exposing the patient to the risk of delayed engraftment. Following the infusion of marrow patients will be treated with

250$\mu g/m^2$/day of GM-CSF until the white cell count exceeds 1,000 as is standard procedure. Harvested autologous peripheral blood stem cells will also be given as support if necessary.

Both peripheral blood and marrow of all patients transduced with the MDR gene will be tested for the presence of MDR DNA and RNA by PCR and Southern blot analyses. In addition, we will use FACS sorting with the 17F9 antibody (32) to determine the percent of transduced cells in which the MDR gene is expressed. We will determine the cell populations (granulocytes or lymphocytes) containing and expressing the MDR gene in peripheral blood and in marrow samples using FACS sorting with antibodies specific for these populations as well as gating on the basis of size and morphology (32). Aliquots for analysis of CFU-GEMM and BFU-E colonies will also be collected and colonies resistant to colchicine measured. Individual colonies will be analyzed for transduction by MDR PCR.

We will use the outpatient facilities of the General Clinical Research Center for follow-up visits for patients treated at CPMC. Marrow will be obtained from patients at 3-6 month intervals for six months posttransplantation and then at six-month intervals thereafter. We will draw blood monthly after transplantation to determine the presence and expression of the MDR gene. At each visit, we will examine patients for progression of their underlying malignancy and for other manifestations potentially resulting from the retrovirus.

Safety tests will be done following human gene transfer into bone marrow. Transduced bone marrow cells from all patients will be cocultured with Mus-dunni cells and supernatants assayed for the presence of reverse transcriptase and in the S+L assay. Serum from MDR-transduced patients will be plated on untransduced 3T3 cells and colchicine resistance of these cells tested (16,17,32,47). In addition, the presence or absence of the MDR gene by PCR will be analyzed in the recipient 3T3 cells after exposure to the serum. Serum from patients as well as transduced cells will also be assayed periodically by co-culture with mus dunni cells and S+L assays.

A potential theoretical risk in this protocol is that undetected residual cancer cells in the patient's marrow will be transduced with the MDR gene and become resistant to MDR-responsive drugs such as taxol. This is more often true for breast cancer than for ovarian or brain cancer. It is possible that these rare cells could become the predominant tumor population and lead to disease relapse and death. While these events might all occur, we do not believe they represent a significant risk to these patients. First, the number of residual cells in the marrow will

be small since they will be undetectable by morphologic and cell sorting techniques in order for the patient to qualify for the protocol. Secondly, even if transduced with the transferred MDR gene, these residual cells would also require the proliferative and other biological characteristics that could make them the precursors of a relapsing population of tumor cells. Thirdly, with treatment with MDR-responsive drugs, either prior to chemotherapy or after exposure to taxol posttransduction, a population of tumor cells with high levels of endogenous MDR may well be selected since this is a common mechanism of drug resistance to tumors. This is indeed the rationale for treating breast cancer with agents other than MDR-responsive agents such as alkylating agents including melphalan and cyclophosphamide, and carboplatinum, thiotepa, 5-fluorouracil and nitrosoureas. In the case that this protocol led to the proliferation of an MDR-transduced residual breast cancer cell population in the marrow, treatment with combinations of these non-MDR responsive drugs will be used as would be the case with MDR resistance in a non-transduced patient. We will monitor the MDR content of marrow and tumor cells both pre- and post ABMT by measuring transferred MDR genes by PCR and MDR expression by FACS sorting in marrow specimens and all available tumor samples, respectively.

6. HUMAN SUBJECTS:

With this protocol, we will embark on human studies in which the MDR gene is transferred into autologous human bone marrow cells prior to reinfusion back into these patients. The use of retroviral gene transfer into lymphocytes of patients has been reported and has demonstrated no toxic side effects to date (76-78). We will evaluate the safety of gene transfer by looking for the presence of intact retrovirus in the bone marrow of MDR-transduced patients. This is the major risk of these studies. In addition, we will use the assays for human MDR at the DNA, RNA, and protein levels described earlier to determine the extent of expression of the MDR gene in human bone marrow cells *in vitro* and eventually *in vivo* in human subjects.

A. Detailed Description of The Proposed Involvement of Human Subjects:

The initial patients whose cells will be transduced with the MDR gene will be those undergoing ABMT for advanced cancers not involving the bone marrow. In these patients, MDR gene transfer and expression and potential toxicity will be assessed. In breast and ovarian cancer patients subsequently receiving high-dose adriamycin or taxol, the potential effect of MDR expression in reducing bone marrow toxicity will be measured.

The goal of initial experiments will be to look for any side effects, to quantitate the number of bone marrow stem cells transduced with the MDR gene both before and after MDR gene transfer, and to evaluate MDR expression and whether selection of MDR-transduced cells occurs after subsequent exposure to the taxol.

The patients used will have no preferential ethnic background, and it is anticipated that in the first studies only adults will be used. In ABMT at CPMC to date, the demography of ABMT patients reflects that of the patients seen at CPMC in general. The bone marrows harvested since June 1990 at CPMC are comprised of 67% female, 10% black, and 10% hispanic. The bone marrow transplants done since July 1990 are comprised of 80% women, 20% black, and 12% hispanic. The preponderance of women is due to transplanting patients with breast and ovarian carcinomas which comprise 40% of the transplants done at CPMC. The New York City population in general is ~10% hispanic and 25-30% black. The health status of these patients will depend on their underlying oncology condition and it is anticipated that transduction of the bone marrow of these patients will have no detrimental effect on their health status.

B. Sources of Research Material:

As indicated in #1 above, patients' bone marrow cells will be analyzed for the presence and expression of the MDR gene at various times subsequent to the initial reinfusion of marrow. The additional number of bone marrow aspirations, if any, required of these patients will be low, perhaps 1-3. Primarily, peripheral blood samples will be used to monitor the survival of transduced cells and MDR gene expression; blood will be obtained from these patients periodically and examined by PCR for the presence and expression of the MDR gene and and by FACS analysis for MDR protein and for intact retrovirus. The peripheral blood sampling may or may not require venipunctures in addition to those ordinarily being performed in the course of monitoring these patients.

C. Recruitment of Subjects and the Consent Procedures to be Followed:

As described above, initially subjects undergoing ABMT in association with intensive chemotherapy for advanced cancer not involving the bone marrow will be used. The investigational aspects of the gene transfer into bone marrow cells will be completely explained to the patients as a procedure that:
1) Initially offers them no significant advantage;

2) Has a small risk of viral infection and an even smaller risk of developing a new cancer. The risk is no greater than that of conventional chemotherapy, and no cases of retroviral infection or cancer have occurred in previous clinical trials; 3) Eventually might allow their bone marrow to be more resistant to the effect of chemotherapeutic agents enabling further chemotherapy to be administered without repeated ABMT and with less marrow side effects; and 4) May eventually lead to new therapy for a variety of disease states. A consent form indicating these considerations is included at the end of this section as part of the protocol.

D. Potential Risks:

The major potential risk of this procedure is that the transfer of retroviruses into the bone marrow cells of patients will alter the normal function of repopulating bone marrow. To date, no such untoward effects have been demonstrated when genes are introduced into or humans (76-78) by retroviruses. Especially important in the safety of this procedure is that no intact retroviruses capable of producing productive retroviral infection are administered. The packaging lines used in the proposed experiments has been demonstrated to be safe in animal studies (16,17). Safety of the MDR transducing retroviral producer line A12M1 will be assessed in transduced human bone marrow cells: 1) In immunodeficient mice given these cells; 2) In fresh marrow cells using coculture with Mus-dunni cells and naive 3T3 cells and subsequent testing of supernatants for reverse transcriptase and MDR by PCR; and 3) Exposing these supernatants to PG4 cells and performing the S+L assay.

E. Confidentiality:

In addition to the description of the cautionary measures indicated in #4 above, confidentiality of the identity of the patients will be maintained by the physicians involved in the protocols. In the event of adverse effects, appropriate medical attention will be administered immediately. These subjects will be carefully monitored by repeated blood count analysis and intermittent bone marrow collection to determine the safety and efficacy of retroviral gene transfer.

F. Risk/Benefit Ratio:

The risks in these experiments appear to be quite low on the basis of other retroviral gene transfer experiments already reported from the NIH and currently underway (76-78). The anticipated benefit to the subjects is not significant in the short run; in the long run, if successful, the use of the MDR

gene as a selectable marker *in vitro* and *in vivo* may lead to its use in protection of bone marrow cells from the toxicity of certain chemotherapeutic agents, which are inactivated by the presence of the MDR gene. These experiments may also provide insights into the use of the MDR gene as a selectable marker to increase the number of cells that also contain and potentially express a second non-selectable gene present in the retroviral vector such as MHC or Il-2.

7. CONSULTANTS/COLLABORATORS:

We will continue to collaborate with Dr. Stephen Goff (Columbia) who is an expert on retroviral vector design, retrovirus infection, and producer and packaging lines.

Dr. John Dick (University of Toronto) will collaborate with us on studies involving the establishment of the bg/nu/xid immune-deficient mouse system and will provide breeding pairs of these mice.

Drs. Michael Gottesman (National Cancer Institute), Ira Pastan (National Cancer Institute), and Susan Kane (City of Hope Hospital) will collaborate with us on studies using MDR retroviral vectors, PHaMDR1/a, and novel vectors being constructed.

Drs. Peter Wiernik (Montefiore) and Subash Gulati (Sloan-Kettering) will collaborate with us on studies involving clinical trials detailed in this grant with the MDR and MHC genes.

Letters of collaboration are attached for each of these collaborators.

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2) CONSENT FORM

The purpose of this consent form is to provide you with the information you need to consider in deciding whether to participate in this research study.

STUDY TITLE: ADDITION OF HUMAN MDR GENES TO BONE MARROW CELLS TO PROVIDE RESISTANCE TO CHEMOTHERAPY ADMINISTERED TO PATIENTS WITH ADVANCED CANCER.

IRB STUDY NUMBER:

You are invited to participate in a research study evaluating the effect of transferring a specific human gene to your bone marrow cells. This gene, known as the Multiple Drug Resistance (MDR) Gene, induces cells to become resistant to certain chemotherapy agents such as taxol. The reason for asking you to participate in this study is that you have advanced cancer which has been treated with surgery, radiation and chemotherapy. Your doctor believes that this therapy is not sufficient to control your disease so he has referred you to Dr. Charles Hesdorffer to undergo a course of high dose chemotherapy followed by autologous bone marrow transplantation (ABMT) as a means of again controlling your disease. The introduction and expression of the MDR gene in your bone marrow cells may prevent drugs you recieve later on from injuring your marrow.

Once you undergo a bone marrow harvest for a standard course of high dose chemotherapy you may elect to participate in this study; no additional tests or treatment would be required for you at that time to do so. If you agree to participate, approximately a third of the marrow harvested will have the MDR gene inserted using a technique known as retroviral gene transfer. This technique involves taking a mouse retrovirus, stripping this organism of its capacity to reproduce, and attaching to it the human MDR gene. The retrovirus serves as a carrier only. This process has been evaluated in animals as well as in humans and has been shown to be safe. On completion of the high dose chemotherapy you will have both "normal" marrow as well as the "gene inserted" marrow reinfused. This will avoid the risk of the marrow transplantation being inadequate for your treatment.

Your participation in this study involves the following risks:
1. Although extremely small, it is conceivable that the defective retroviruses we use to transfer new genes to your bone marrow cells may result in harm. In the worst possible case, which has never been reported in humans, this might result in the development of a new cancer. We have done extensive studies in animals that show that the defective retroviruses we use in these studies are extremely unlikely to cause this problem. In addition, it is possible that this treatment may affect cancer cells in your bone marrow thus making them more resistant to certain drugs. However, we will be isolating specific marrow progenitor cells in your bone marrow (CD34 positive cells) prior to introducing the MDR gene in

order to decrease the likelihood of cancer cell contamination. In the unlikely event that your tumor recurs as a result of reinfusion of contaminating gene transduced cancer cells we can always treat you with other non-MDR responsive drugs. It is not known whether the gene transduction process interferes with the recovery of normal bone marrow function. To avoid any possibility of this occurring you will receive sufficient untransduced marrow at the time of marrow infusion to guarantee the success of the transplant.

2. The risks of high dose chemotherapy and ABMT are the same in this study as those if you were not in the study. The major problems relate to the risk of infection which may occur as a result of the ablative effects of the high doses of chemotherapy. In addition, there are toxicities due to the drugs used that affect organs other than the marrow. These are listed in the attached table and involve predominantly the heart, kidneys, liver, bladder and nervous system. In addition, it is likely that you will develop significant nausea and vomiting around the time the chemotherapy is administered although you will receive medications to limit this problem. In order to minimize the risk of infection you will be kept isolated in a room especially equipped with a clean air HEPA filter. Visitors will be limited to no more than two people; both visitors and medical personnel will wear protective clothing, and all food you receive will be specially treated to decrease the possibility of transmitting any infection to you. In addition, you will receive prophylactic therapy for viral, fungal and bacterial organisms. If you are unable to eat you will receive intravenous feeding. Finally, you will receive blood and platelet transfusions in order to maintain a normal hemoglobin and to prevent bleeding.

Six weeks after the marrow is reinfused you will undergo a series of tests designed to evaluate the response of your tumor to the high dose chemotherapy as well as to determine whether the marrow with the added MDR gene is present and is functioning normally. The presence and activity of this gene would indicate that your marrow may be more resistant to certain anti-cancer drugs such as taxol you might receive at this time or later. Further treatment with such drugs is not part of this protocol, and will be left to decisions made by you and your physician at the appropriate time following ABMT.

Almost all of the tests you will undergo would be done even if you did not participate in the study. However, it may be necessary to do an additional bone marrow or blood test to evaluate the results of this new treatment. The following blood tests and marrow samples will be required if you participate in this study:
A. Blood and marrow - 6 weeks after ABMT.
B. Blood weekly after each dose of chemotherapy and blood and bone marrow at 3 month intervals should you receive additional chemotherapy after ABMT.
C. Marrow every 6 months after ABMT or once all other therapy for recurrent disease is discontinued.

These tests are necessary to determine the presence and function of

the MDR gene as well as to determine the status of your bone marrow function after ABMT and chemotherapy.

Chest X Rays and CT Scans will be required at regular intervals to assess the status of your cancer. These tests would be necessary regardless of the treatment you receive.

The benefits to you of your participation in this study may not be immediately apparent or may not occur at all. If you do benefit it may be due to your ability to better tolerate various MDR responsive drugs after bone marrow transplantation should your cancer recur or be incompletely eradicated and if you and your physician decide on instituting further treatment. In addition, this study may provide us with insights into how to use the principle of gene therapy to attempt to help other patients with various tumors and genetic defects. In this way you would be aiding society by providing new scientific data.

Should you decide not to participate in this study, it will not affect your treatment at CPMC including high dose chemotherapy and ABMT without gene therapy. However, there are no specific alternatives to the new approach being offered in this study other than nonparticipation in the study.

The costs of extra blood and marrow tests required for this study will be covered by the study. In addition, any potential complications arising from the gene transduction process will also be covered by the study. You will still be responsible for paying for the transplant and all the regular visits and tests required to care for your cancer. We will attempt to expedite your visits to the physician so that you do not need to spend more time in the hospital than you normally would if you did not participate in this study.

Any information obtained during this study and identified with you will be kept strictly confidential. All information accrued in this study will be released in the form of a number assigned to you to identify you to the investigators involved in the study. In any publications, only these numbers will used. There will be no need at any time during the course of this study for disclosure of your participation other than that associated with your informed consent.

Your participation in this study is completely voluntary. You can withdraw from or refuse to participate in the study at any time without prejudicing your medical care at Columbia Presbyterian Medical Center.

You will be given sufficient time to consider your participation in this study. Should you have any questions you can call Dr. Hesdorffer at 212-305-1738 and he will meet with you in order to answer any further questions you may have. If you have questions as to your rights as a research subject, you may call the Institutional Review Board at 212-305-6950.

CONSENT TO PARTICIPATE IN THE STUDY

I have discussed this study with Dr. Hesdorffer to my satisfaction. I understand that my participation is voluntary and that I can withdraw from the study at any time without prejudice. I have read the above and agree to enter this study. Signing this form does not waive any of my legal rights.

I have been informed that if I believe that I have sustained injury as a result of participating in this research study, I may contact the principal investigator, Dr. Hesdorffer, at 212-305-1738 or the Institutional Review Board at 212-305-6950, so that I can review the matter and identify the medical resources that are available to me.

I understand that:

a) The Presbyterian Hospital will furnish any emergency medical care determined to be necessary by the medical staff of the hospital.

b) I will be responsible for the cost of such care, either personally or through my medical insurance or other form of medical coverage.

c) No monetary compensation for wages lost as a result of injury will be paid to me by Columbia Presbyterian Medical Center.

d) I will receive a copy of this consent form.

Signature: Participant

Witness

Investigator

Date

The solicitation of subjects into this study has been approved by the Columbia Presbyterian Medical Center Institutional Review Board.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
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RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experienc and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and cor plete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NC EXCEED TWO PAGES.

1978	ECFMG CERTIFICATE
1979	INTERN IN MEDICINE AND SURGERY JOHANNESBURG TEACHING HOSPITAL, SOUTH AFRICA
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1984	CHIEF RESIDENT, MEDICINE, HEMATOLOGY, ONCOLOGY JOHANNESBURG TEACHING HOSPITAL, SOUTH AFRICA
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1986-1989	HEMATOLOGY-ONCOLOGY POSTDOCTORAL FELLOW, DEPT. OF MEDICINE COLUMBIA UNIVERSITY
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1989	NIH NRSA FELLOWSHIP AWARD.
1990	MATHESON FOUNDATION ABMT AWARD.
1990	ACS INSTITUTIONAL GRANT AWARD.

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FF PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR Karen H. Antman, M.D.

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Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

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INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Muhlenburg College, Pennsylvania	B.S.	1970	Chemistry
Columbia University College of Physicians and Surgeons	M.D.	1974	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1974-75 Intern in Medicine, Columbia Presbyterian Medical Center, NY
1975-77 Resident in Medicine, Columbia Presbyterian Medical Center, NY
1977-78 Clinical Fellow in Medical Oncology, Sidney Farber Cancer Institute,Boston, MA
1977-78 Clinical Fellow in Medicine, Harvard Medical School, Boston, MA
1977-79 Assistant in Medicine, Brigham and Women's Hospital, Boston, MA
1978-79 Research Fellow in Neoplastic Disease Mechanisms, Sidney-Farber Cancer Institute, Boston, MA
1979-82 Junior Associate in Medicine, Brigham and Women's Hospital, Boston, MA
1979-83 Instructor in Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA
1979-83 Clinical Associate, Dana-Farber Cancer Institute, Boston, MA
1982- Associate Physician, Brigham & Women's Hospital, Boston, MA
1983- Assistant Physician, Dana-Farber Cancer Institute, Boston, MA
1983- Assistant Professor of Medicine, Dana-Farber Cancer Institute and Harvard Medical School
1985- Assistant in Medicine, Beth Israel Hospital, Boston, MA
1988- Associate Professor of Medicine, Dana-Farber Cancer Institute and Harvard Medical School

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1979 ABIM, Subspecialty Certification in Medical Oncology # 059247

Publications:

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Lew MA, Peters WP, Schryber S, Antman K, Eder JP, Andersen JW, Levin MJ, Finberg R. Prophylaxis of bacterial infections with ciprofloxacin in patients undergoing autologous bone marrow transplantation. Transplantation (in press).

Lois J. Ayash, M.D., Myla Hunt, M.S., Karen Antman, M.D., Lee Nadler, M.D., Catherine Wheeler, M.D., Tak Takvorian, M.D., Anthony Elias, M.D., Joseph H. Antin, M.D., Thomas Greenough, M.D., J. Paul Eder, M.D. Hepatic Veno-occlusive Disease in Autologous Bone Marrow Transplantation of Solid Tumors and Lymphomas, J Clin Oncol, 1990, 8:1699-1706.

Antman K, Elias A, Hunt M, Ayash L, Anderson K, Wheeler Cm, Schwartz G, Tepler I, Mazanet R, Critchlow J, Schnipper L, Demetri G, Griffin J, Frei E. GM-CSF potentiated peripheral blood progenitor cell (PBPC) collection and use after high dose chemotherapy. Blood 1990; 76:526a.

Mazanet R, Elias A, Hunt M, Ayash L, Anderson J, Wheeler C, Schwartz G, Tepler I, Critchlow J, Schnipper L, Demetri G, Griffin J, Frei E, Antman K. Peripheral blood progenitor cells (PBPC)s added to bone marrow (BM) for hemopoietic rescue following high dose chemotherapy for solid tumors reduces morbidity and length of hospitalization. Proc Am Soc Clin Oncol 1991; 10:1140.

HUMAN GENE TRANSFER PROTOCOL

9306-052 Ilan, Joseph, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio; *Gene Therapy for Human Brain Tumors Using Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I.*

Date of RAC Approval: 6/8/1993
Date of NIH Approval: 12/2/1993

ABSTRACT

The following protocol represents a treatment approach to otherwise incurable malignant human brain tumors that is based on an antisense gene therapy strategy. This antisense strategy has been used to cause complete regression of malignant gliomas in rat brain. We and others have shown that the C6 glioma cell line produces an overabundance of insulin-like growth factor (IGF-I). We designed an episome based vector to produce multiple copies of itself when inside of the cell, and to express RNA that is anti-sense to IGF-I. Using this vector, we have demonstrated a complete suppression of IGF-I synthesis in transfected C6 glioma cells. Injection of the transfected cells subcutaneously into rats bearing C6 glioma tumors results in complete regression of tumor. This occurs whether the tumor is within the cranium or in an extracranial site at a point distant to the site of injection of the transfected cells. We have further shown that the mechanism which results in destruction of tumor includes the promotion of a CD8 T cell-mediated immune response which is selective for the glioma cells. In mixed tumors containing both C6 glioma and neuroblastoma, the T cell driven immune response remains targeted selectively to the glioma cells. This strategy of treatment is proposed in the protocol which follows. First, we will select patients with gliomas which over-express IGF-I. Second, these patients will be treated with the anti-sense strategy using the episome based vector. Finally, in the first phase of this trial we will determine toxicity which may define the limits of use for this methodology. The second phase of this study will determine efficacy as defined by changes in survival, time to tumor progression and/or to tumor regression.

This approach to gene therapy for human brain cancer has several unique advantages. First, the episome based vector includes component parts of a virus that commonly infects humans and although capable of driving extrachromosomal episomal replication, does not reproduce active virus. Second, the large copy number of the vector within transfected cells insures the expression of antisense RNA quantities sufficient to inhibit the production of IGF-I. Third the technique will not require stereotactic injection into the site of malignancy, hence surgically invasive procedures over and above those ordinarily used to diagnose and treat such brain tumors will not be needed. Finally. the mechanism for tumor distruction includes a selectively targeted T lymphocyte mediated immune response leading to the prediction that complicating toxicity should be low.

PROPOSAL FOR A PHASE ONE GENE THERAPHY CLINICAL STUDY

Project Title

Gene Therapy for Human Brain Tumors Using Episome-based Antisense cDNA Transcription of Insulin-like Growth Factor I

Names of investigators:

Principal Investigator:
Joseph Ilan, PhD, Dept. of Pathology (216/368-3590)

Co-Principal Investigators:
Robert A. Ratcheson, M.D., Chief Neurosurgery, University Hospitals
Donald D. Anthony, M.D., Ireland Cancer Center, Dept. of Pharmacology
Mark L. Tykocinski, M.D., Dept. of Pathology

Associate Investigators: Neurosurgery
Robert Fenstermaker, M.D.
Edward Ganz, M.D.
Timothy B. Mapstone, M.D.

Associate Investigators: Gene Therapy and Immunology
Judith Ilan, PhD
David Kaplan, M.D. PhD
Thomas R. Johnson, PhD

Associate Investigator: Neuropathology
Pierluigi Gambetti, M.D.

Institutions: Case Western Reserve University School of Medicine and University Hospitals of Cleveland.

Estimated Duration of Study: 3 years

Number and Kind of Subjects:	Number	Sex	Age
Patients with Glioblastoma multiforme	12	M & F	> 18

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II. Research Plan

A. Specific Aims and Hypotheses

1. To describe toxicities, if any, and to demonstrate the safety of subcutaneous injection of autologous in vitro cultured, transfected and irradiated glial tumor cells in patients with glioblastoma multiforme.

2. To demonstrate the efficacy of injection of autologous in vitro cultured, transfected and irradiated glial tumor cells in destruction of native tumor and/or in delay to time of recurrence.

We plan to develop a research program that is expanded beyond these phase I toxicity studies. Experiments would be designed to learn if effective down-regulation in the expression of IGF-I in transfected human glioma cells will result in up-regulation of the T cell mediated immune response similar to that which occurs in the rat model. The experimental approach would also be designed to address the question of whether the immune response is directed towards both transfected and non-transfected human glioma and ultimately to whether it leads to tumor regression and increased length of survival.

B. Introduction

-Clinical Background

Malignant glioma Each year in the United States approximately 17,500 patients are newly diagnosed with malignant brain tumors(1). Malignant gliomas cause 2.5% of all deaths due to cancer and are one of the leading causes of death from cancer in young adults, 15 to 34 years of age (2,3). The prognosis for patients with such malignant tumors remains uniformly dismal. The length and quality of survival have improved only minimally over the past 20 to 25 years despite developments in neurosurgical technique, radiotherapy and chemotherapy (4). Survival in patients with malignant gliomas (glioblastoma and anaplastic astrocytoma) with current therapy is 40 to 50 weeks. 5). Radical surgery and radiotherapy remain the cornerstone of treatment (6,7). Patients undergoing gross total resection and radiotherapy have a median survival of 12 months. Thirty-four percent survive 18 months or longer (5,8). Radiation directed to the tumor and the surrounding area also prolongs survival (5). A very modest contribution is added by conventional chemotherapy (9,10,11,12)

Glioblastoma multiforme is the most invasive of the astroglial neoplasms with a tendency to spread widely throughout the brain. Glioblastoma is very often extensively spread by time of diagnosis, leading to unresectability, inadequate local control and a consistently poor prognosis. Median survival is less than one year after diagnosis, and 5 year survival is less than 5.5% for this tumor (2,13). Clinical features which correlate with improved prognosis include an age of less than 65 years and a high Karnofsky performance rating at the time of diagnosis (see Attachment A). Although malignant glioma remains a fatal disease, significant advances have been made in the treatment of other malignant central nervous tumors such as medulloblastoma. Increased recruitment into clinical studies will be needed if similar progress is to be made in the treatment of gliomas(14).

Pathologic classification Gliomas most commonly arise by malignant transformation of astrocytes. Prognosis is largely determined by pathologic features. The World Health Organization (WHO) system of classification divides these tumors into low -grade astrocytoma (low grade gliomas), anaplastic astrocytoma and glioblastoma(high grade gliomas(3,15). The Daumas-Deport grading system uses four critieria based on nuclear atypia, mitoses, endothelial proliferation and necrosis. The system of grading used at the division of neuropathology of CWRU is a modification of these previous systems.

WHO Grade	Our Designation	Description
1	Pilocytic astrocytoma	- Astrocytoma comprised predominantly of piloid cells with or without a biphasic pattern. Endothelial proliferation often present, as are Rosenthal fibers and protein granular bodies. Mitoses only within endothelial cell population. Necroses absent.
2	Astrocytoma	Diffuse increase in non-reactive astrocytes (high nuclear/cytoplasmic ratio) with mild to moderate atypia. Clusters of undifferentiated tumor cells, mitoses, endothelial proliferation & necrosis are absent.
3	Anaplastic Astrocytoma	Features of astrocytoma, plus clusters of undifferentiated tumor cells, marked nuclear and cytoplasmic pleomorphism and mitotic figures. Endothelial proliferation and necrosis absent.
4	Glioblastoma Multiforme	Features of anaplastic astrocytoma plus endothelial proliferation and/or necrosis.

The distinction between low grade and high grade gliomas is important for determining prognosis and treatment since patients with low grade astrocytomas often have a mean survival time measured in years and are frequently treated by surgery alone. Patients with high grade gliomas have a mean survival time of less than a year and failure is due to tumor progression despite aggressive therapy. Although patients with anaplastic astrocytoma may have a more variable course than those with gioblastoma, ultimately recurrence or progression to glioblastoma multiforme is the rule.

-Research Background and Rationale for Study

Tumor Immunology Tumors produce remote effects on the immune system which promote growth of the tumor. Mechanisms which have been invoked to explain these effects include production of suppressor factors by the tumor and diminished production of cytokines (17,18). Malignant gliomas (which develop and grow locally in the brain) are partially sequestered from the immune system, yet they are associated with marked systemic suppression of humoral and cell-mediated immunity (19). Peripheral blood lymphocytes from patients with glioma respond poorly to mitogens and/or antigens, and the percentage and absolute numbers of peripheral blood T cells are decreased (20). IL-2 production by lectin stimulated T cells is significantly less than that by T cells from normals. Activated T cells from patients with glioma have a defect in the expression of high affinity IL-2 receptors. A defect in helper-cell function may also explain the known clinical failure of glioma patients to mount antibody responses to influenza virus and tetanus toxoid. A T cell suppressor factor present in the sera of patients with glioma has been described (21). More recent work isolating and characterizing glioblastoma-derived T cell suppressor factors have identified the TGF-βs as a mediator of this action. TGF-β_2 which is elaborated by malignant gliomas has been shown to inhibit T cell proliferation in vitro by IL-1 dependent mechanisms (22,23). Immunosuppressive mediators produced by glioma cells in culture induce cellular and biochemical dysfunction in immune cells (24). RNA for TGF-βs have been identified in most glioblastomas (25).

Efforts to enhance immunity as a way of treating astrocytoma and glioblastoma have not been effective, possibly because of immune suppression produced by the tumor (26). Partial amelioration of T cell suppression, after surgical resection of the glioblastoma has been observed clinically (27). A variety of strategies have been tried, from non-specific enhancement of the immune response by interferon and interleukin-2, to injection of activated lymphocytes (17) and monoclonal

antibodies. Several workers have attempted to produce active immunization by subcutaneous injection of autologous glioma cells or cultured glioma cell lines (28). In this study 20 patients were injected with irradiated human glioma cell lines (not autologous) along with 500 mg of bacille Calmette-Guerin cell wall. A trend to increased survival with inoculation was described. Importantly, no allergic encephalomyelitis resulted (29).

Previous Work with IGF-I and Glial Tumors Many tumor types express high amounts of IGF-I or the related growth factor IGF-II (30). The expression of IGF-I and IGF-II genes is markedly enhanced in glial tissue from glioblastoma and anaplastic astrocytoma as compared to normal adult brain (31). This over-expression of selective growth factors including IGF-I and II has been implicated in uncontrolled growth and maintenance of the tumor phenotype. (32,33). The expression of IGF-I and II is also high in fetal brain. Thus the expression in tumors of genes that are active during embryonic or fetal development could represent re-enactment of an early embryonic or fetal program, enabling the cells to in some way evade immune recognition (34). Most data bearing on this point have been obtained in reference to **IGF receptor** status. IGF-I binding was demonstrated by competition-inhibition binding assays in plasma membrane preparations from meningiomas, glioblastomas, and normal adult brain. High affinity 125IGF-I binding was greatest in gliomas and in meningiomas,and minimal in normal adult brain (35). Immunohistochemistry staining using mouse monoclonal antibodies to IGF binding protein has demonstrated this protein intracellularly in 4 of 6 gliomas (36). Scatchard analysis demonstrated increased specific IGF-I binding to cell membrane preparations from 18 gliomas (33).

Other experimental data address the **relative expression of IGF** genes. We and other have shown that the rat C6 glioma expresses high levels of IGF-I, produces large quantities when grown in serum-free medium, and forms rapidly growing tumors in syngeneic animals. In addition, when examined by immunocytochemical techniques, high levels of IGF-I expression were detected in 20 of 20 fresh frozen human glioma samples (Ilan, unpublished observation).

In studies by others the expression of both IGF I and IGF II were increased in comparison to normal brain tissue in one series of 10 freshly obtained human astrocytomas including 2 grade IV glioblastomas (51), and in a second series of 4 brain cancers including 3 glioblastomas and 1 anaplastic astrocytoma (31). However, in a study that evaluated relative transcription rates for a number of growth factors in cultured cells, many of which were established cell lines, results were less clear. Both IGF I and IGF II were highly expressed in some glioblastoma cell lines but a uniform pattern of overexpression was not obtained (52). The results are consistent with findings by Glick et. al. who reported no significant production of IGFs by specific radioimmunoassay in tumor cell conditioned serum free media of 12 glioblastomas (53). However, in our experience the cellular characteristics of cultured glioblastoma cells differ significantly after several cell passages in comparison to those of fresh cells. Moreover, IGF protein is not secreted and/or not retained in stable form within the cell conditioned media, but rather accumulates within the cell (see Fig 3, ref 34).

Previous studies have looked for a role of **IGF-I** and IGF-II in driving proliferation of tumor cells. Growth in animal models has been blocked with specific antibodies to IGF (37,38). We chose to block IGF-I expression through the introduction of antisense IGF-I cDNA into C6 rat glioma cells. An episome-based plasmid, pAntiIGF-I, was constructed that incorporates Epstein-Barr virus replicative signals and the ZnSO4-inducible metallothionein I transcriptional promoter. (See Attachments H, I and J) The transfectants express high levels of IGF-I mRNA and protein when cultured in absence of ZnSO4. Addition of ZnSO4 to culture media resulted in high levels of antisense transcripts and a dramatic decrease in level of endogenous IGF-I mRNA and IGF-I protein within these cells. The subcutaneous injection of these transfected glioma cells into syngeneic rats brought about the induction of an immune response against the transfected glioma cells. Moreover, the induced CD8 T lymphocytes were capable of recognizing and destroying existing tumor even when the transfected cells were injected at a point distant from the tumor, whether localized intra or extra-cranially. We concluded that this IGF-I antisense strategy may reverse a phenotype which allows C6 glioma cells to evade immune surveillance (30).

Previous Human Experience with Gene therapy in Cancer The ability to manipulate and insert genes into human cells has opened new possibilities for treating cancer (39). Initial human trials involved tumor infiltrating lymphocytes (TIL) harvested from resected tumors. Marker genes were added to the TIL to demonstrate the feasibility and safety of using retroviral-mediated transduction of human cells (40). TIL marked with a gene for neomycin resistance were returned to cancer patients and demonstrated to accumulate at tumor sites. The IL-2 dependence of the retroviral-transduced TIL is believed to provide a safeguard against transformation to a malignant phenotype (41). In other studies, TIL have been transfected with the Tumor Necrosis Factor (TNF) gene. When activated, widespread tumor necrosis occurred and remission of disease was reported (41). Viral vectors have certain advantages over physical and chemical methods for introducing DNA into human cells. First a wide range of cell types can be infected. Second the efficiency of the process approaches 100%. However, use of retroviruses in clinical trials continues to raise concerns about safety. One fear has been that recombination of vector sequences with coding sequences in the infected cell line could generate replication-competent viruses. Modifications to the viral genome would minimize this possible risk (42).

A retroviral or herpes simplex vector that replicates only in dividing cells has provided the rationale for two new gene therapy approaches to glioma. An experimental therapy of human glioma using a genetically engineered herpes simplex virus-1 (*dl*sptk), attenuated for neurovirulence has been tested in nude mice. In vitro, the vector destroyed a human glioma line (U87) when added over a wide range of concentrations. In vivo, the injection of the *dl*sptk vector into U87 gliomas in nude mice resulted in improved survival, and total tumor remission in some animals (43). Another approach has used mouse fibroblasts genetically altered with a retroviral vector carrying a herpes gene for thymidine kinase(HS-tk). The fibroblasts were injected stereotactically into C6 gliomas of rats. After 5 days during which the replicating HS-tk retroviral vectors transduced neighboring proliferating glioma cells, the rats were treated with the anti-herpes drug ganciclovir (44). The gliomas underwent complete macroscopic and microscopic regression. This protocol is currently in human trials (45).

C. Study Design

1. Specific Procedures

-Overview of Experimental Treatment Plan

a. Informed consent will be obtained after surgical intervention in patients and histologic confirmation of glioblastoma multiforme.
b. Tumor cells will be obtained at the time of stereotactic brain biopsy for diagnosis, or at the time of open craniotomy for therapy.
c. Cell cultures will be established irrespective of final histology.
d. The expression of IGF-I by the cultured cells will be determined by an ELISA technique.
e. Cultured cells from those tumors which have histopathologic features of glioblastoma multiforme and which demonstrate IGF-I production will be transfected with the episome- based vector containing antisense to IGF-I.
f. Transfected cells will be irradiated with 5,000 cGy and injected subcutaneously into the patient from which they were obtained.
g. Dose calibration. Three groups of four patients each will be treated subcutaneously at each of the following doses of transfected and irradiated cells: $10X10^6$, $50X10^6$, and $200X10^6$.
h. Booster injections of transfected and irradiated cells will be introduced at 4 and 12 weeks.

i. Evaluation for toxicity will take place over the first 48 hours in the Clinical Research Center and three times per week for the first week, then weekly for three weeks. After the second injection, the patient will be again monitored for 48 hours in the Clinical Research Center. Followup will then be weekly for three weeks, every two weeks for four months, and monthly thereafter.

-Acquisition of Tissue, Tissue Culture and Transfection Techniques

Acquisition of Human Tissue and Quality Control of Culture Procedures All human tissue will be obtained in the operating room using standard surgical techniques. Tissue will be transported to a dedicated tissue culture facility in closed, sterile containers. All work with tissue and cells will be done in Category II environmental hoods. Laboratory personnel will wear dedicated laboratory apparel for work with human tissues. All buffers, tissue and culture materials, materials for transfection and for injection into the recipient host will be FDA approved pharmaceutical grade. Quality of cell culture procedures will be monitored as follows:

1. Serum additives will be determined according to specification of the manufacturer and validated by two members of the gene therapy team prior to use.

2. The patient's history of hypersensitivities will be coordinated for possible cross reactivity with antibiotics or other products routinely used in preparation of the transfected cells. The final preparation of cells will be labelled appropriately concerning antibiotics used during selection of transfected cells.

3. During the in vitro handling of patient's cells, preparations will be routinely cultured for bacteria, fungus, and virus.

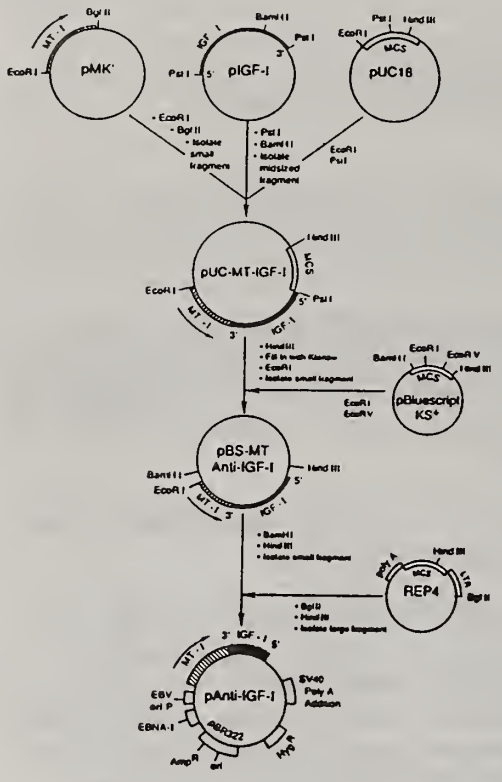
4. Samples from each patient will be verified by both the surgeon and tissue culture technician, and logged into appropriate records as received. Each sample will be color-coded in the log book, and all tissue culture materials used in conjuction with the patient's sample will be similarly color-coded. Prior to innoculation into the patient, thelog record and color coded data will be reviewed and verifed by two independent team members.

Human Glioma Primary Cell Culture Protocol Primary cultures are derived according to the techniques as described by Manfred and Westphal with some modifications(46). At surgery, tissue for culture is placed in serum-free Dulbecco's Modified Eagle's Medium (DMEM) at room temperature and transported to the culture facility. All cell culture is performed in a Category 2 Environmental Laminar Flow Biologic Safety Cabinet. Between 0.5-1 g of tissue is placed in a 100 mm dish and minced finely with sterile iridectomy scissors in calcium and magnesium-free phosphate buffered saline. This medium is replaced with 10 ml of the same medium to which had been added 0.05% Pronase (Calbiochem), 0.03% collagenase (Worthington C1sII), and 0.01% Worthington DPFF DNase I. The dish is placed on an orbital shaker at 37° for 20 minutes. The suspension is then dispersed with a sterile Pasteur pipette, then in a sterile 10 ml serological pipette. At this point the cell number is estimated in a hemocytometer. The cell suspension is spun down in a table top centrifuge at approximately 250 g for 4 minutes. The supernatant is decanted, the cells are resuspended in DMEM containing 10% fetal bovine serum and distributed to 60 mm Nunc tissue culture plates (some coated with fibronectin) at a cell density of about 2X 106 cells/dish. The cells are allowed to attach for 24 hours before medium change. After attachment and cell division are verified but

before cell confluence, the cultures are enriched for glial elements by exposure to serum free DMEM (47) for a period of 5 days with two medium changes to remove detached cells. The cells are then tranferred to 75 cm2 culture flasks (Gibco) for standard trypsinization methodology.

Human Glioma Transfection Protocol We have described the transfection of rat glioblastoma cells by the episome based vector (30,34). Since these early animal studies, we have had experience in transfecting this episome into cultures derived from anaplastic human astrocytomas as well as glioblastomas. These tumors were obtained as discarded tissue from surgery at University Hospitals of Cleveland. After a week the cells are subcultured and transfected with the episome-based plasmid containing antisense to IGF-I.

The construction of this plasmid was as previously described and as depicted in the following schematic.



This antisense expression vector is episomal and includes the Epstein-Barr virus origin of replication and the gene encoding nuclear antigen I, which together drive extrachromosomal replication. The vector replicates readily within human cells providing a copy number of up to 100 copies/cell(48). This allows for a high level of expression of anti-sense IGF-I RNA within the cells. The anti-sense IGF-I cDNA is linked to the highly inducible mouse metallo-thionein promotor. Downstream of the antisense DNA is a polyadenylation termination signal. The construct also contains genes for both hygromycin and ampicillin resistance.

Cells are transfected using the Lipofectin reagent kit available from Gibco BRL according to the manufacturer's instructions. The method is based on encapsulation of the DNA within cationic liposomes which are then fused to the cells' surface. Essentially, 1-5 µg of DNA is mixed with the Lipofectin liposome suspension and applied to actively growing cells in low serum culture medium (Opti mem; Gibco-BRL). Following overnight incubation, the liposome-containing medium is aspirated and replaced with normal growth medium. After an additional 48 hours of incubation, hygromycin B is added at a concentration (which must be established for each cell line) previously determined to kill untransfected cells over the course of about a week. The cultures are monitored until only actively growing colonies are present, at which time they are transferred to flasks and propagated in the continuous presence of hygromycin in order to maintain selective pressure.

Using this technique, stable transfectants of the human glioblastoma or astrocytoma cells were derived. The addition of zinc sulfate to the culture media of the transfected cells activate the MT1 promoter that drives the expression of the antisense IGF-I RNA. This expression was demonstrated by RNA transfer blot hybridization (Northern). The activation of this promoter by zinc sulfate resulted in

complete disappearance of IGF-I transcripts shown by Northern blot hybridization as well as IGF-I protein as determined by immunocytochemistry with antibody to IGF-I and according to techniques previously demonstrated for the rat glioblastoma (32,36).

Preparation of Transfected Tumor Cells for Reinjection To insure quality control appropriate for the cell material to be reinjected subcutaneously, the following tests will be done:

1. 48 hours prior to clinical use, sterile cultures for routine bacteria and mycoplasma and endotoxin levels will be obtained and assayed in the bacteriology and virology laboratories of University Hospitals.

2. On the day of harvest of the transfected cells, the cells will be trypsinized and placed into 50 ml conical tubes (Falcon) for centrifugation. An aliquot of supernatant will be obtained

3. In order to remove materials from cells prior to reinjection, the transfected cell preparations will be washed and pelleted X 3 in 250 ml volumes of phosphate buffered saline pH 7.40. Viable cell count will be determined by exclusion of Trypan blue. The final preparation of cells will be suspended in preservative free normal saline for injection at a concentration of 50 million cells per ml.

-Patient Procedures, Conventional Treatment, and Time Schedule

Patient Groups. All patients will have undergone biopsy and/or resection of their supratentorial Glioblastoma multiforme as deemed appropriate by their attending neurosurgeon at the time of entry into the study. The experimental treatment will not begin until after radiation therapy (if prescribed) is complete. Patients will have been weaned from any high potency glucocoricoids to the extent possible as dicated by their residual tumor and cerebral edema. The first treatment will take place at about 6 weeks after the original surgical intervention. The patients will be consecutively divided into three experimental groups consisting of four patients each. Each patient will receive his or her own transfected and irradiated cells injected subcutaneously over the left forearm. Four patients will receive $10X10^6$ transfected cells; four, $50X10^6$; and four, $200X10^6$. The patient's glioma cells will be maintained in continuous culture and additional booster doses will be injected in the opposite forearm alternately at one and three months using the same initial dose. At six months assuming acceptable toxicity, patients would be entered into a randomized study of treatment efficacy.

Clinical Observation. Patients will be admitted to the Clinical Research Center (CRC) at University Hospitals six to eight weeks following surgery and on the day prior to experimental treatment. They will stay in the CRC for the first 48 hours after reinjection of the transfected and irradiated tumor cells. In that setting frequent monitoring of vital signs and temperature will be performed looking for evidence of systemic effects. Patients will then be discharged to outpatient followup. Each will be given a thermometer and asked to record oral temperatures twice daily while at home over the six weeks after the injection. Each will be seen on the Clinical Research Center for blood work and brief physical examination three times in the first week then weekly for three weeks.

General Physical Examination A complete medical history and physical examination, including detailed neurologic exam and examination of the lymph nodes will be performed on each study subject upon signing consent for the trial before surgery, at the start of the trial (after completing the usual radiation treatment protocol). Evaluation for toxicity will take place over the first 48 hours in the Clinical Research Center and daily for the next week, followed by weekly for a period of three weeks. After the second injection, patient will be again monitored for 48 hours in the Clinical Research Center. Follow up will then be weekly for three weeks, every two weeks for four months and monthly thereafter.

Local Injection Site Special attention will be given to measuring and monitoring the forearm site of injection for evidence of tumor growth, inflammation, or infiltration. Injection sites will be graded on a simple 0 to 4+ scale for evidence of inflammation or ulceration (See Attachment C). Local wound care will be provided if indicated. Since injected cells will have been irradiated, growth of a tumor mass at the injection site will be very unlikely. However, the original injection site in the forearm will be biopsied at four weeks and examined histologically for evidence of tumor reocurrence or (as expected) infiltration with immune cells.

Neurologic Examinations. Although no direct manipulation of neural tissue is involved, it is possible that an immune-mediated response to the tumor could produce peri-tumoral cerebral edema with transient or permanent neurologic deficit. Such a reaction is likely to occur between one and four weeks following injection. Therefore patients will be evaluated with serial neurologic and physical exams. Results of these exams will be compiled and maintained in a file by the data coordinator for this study. Patients and their families will be advised regarding neurologic changes for which they should call the attending neurosurgeon. Any report of decreased neurologic function will be cause for immediate neurologic examination and repeat MRI scan. In addition, followup MRI scans will be obtained regardless of neurologic function at 8 weeks and again at 4 months visit. The examining physician will be looking for evidence of infection or activation of Epstein/Bar infection and will be especially alert to any evidence of fever or neurologic deterioration which could signify autoimmune demyelinating encephalitis. If the latter is suspected appropriate diagnostic and clinical managment steps will be offered, including rebiopsy and increased glucocorticoid therapy if necessary.

Performance Ratings. Psychometric testing and scoring using a Karnofsky performance score will be performed before surgery, at the start of the trial, at four weeks, then every other month. In addition to the Karnofsky functional ratings (See Attachment A), patients will be evaluated in the Neurooncology clinic using the Levin scale (Attachment B; 49). These ratings will be applied to major categories such as area of tumor enhancement on MRI, mental status, motor function, seizure frequency, sensory abnormalities, gait, and overall clinical impression.

Laboratory Studies: The following laboratory evaluation will be performed when a patient is entered into the study: CBC with differential, Epstein-Barr virus titer, HIV antibody screen, CD4/CD8 ratio, serum immunoglobulin levels and a routine chemistry panel including BUN, creatinine, and liver function studies (SMA-18). At each visit described above the following will be repeated: CBC with differential, C4/C8 suppressor cell ratios, routine chemistry panel. Patients will receive skin testing with a panel for delayed type hypersensitivity (Tb, Mumps, Candida, Trychophyton Diptheria Toxin, and control) before surgery and at the end of the study (six months). Chest X-Ray will be performed at the time of admission and at 4 months.

NeuroRadiologic Studies Magnetic Resonance Image (MRI) scanning with and without Gadolinium contrast will be performed as part of routine management in these patients before surgery and after completion of radiation therapy (also the beginning of the clinical trial). Patients will be evaluated with pre- and post-procedure MRI. MRI will be repeated at 8 weeks and 4 months. Chest X-Ray will be performed at the time of MRI.

Other Studies. As part of this study the patient will be asked to undergo phlebotomy of 50 ml of whole blood at four to six weeks after surgery. Cells will be centrifuged and the buffy coat layer of white blood cells retrieved. Culture of glial tumor cells will be divided into two portions. One portion will be transfected with plasmid containing antisense to IGF-I, and stable transfectants will be derived by selection with the antibiotic Hygromycin®. The other portion of glial cells, derived from the parental culture, will serve as control. Both a culture of transfected cells as well as the non-transfected line will be irradiated with 5000 cGy from a cobalt source. This treatment has been shown to result in cells that do not divide and do not incorporate tritiated thymidine into DNA. The irradiated and transfected culture will be co-incubated with the buffy layer containing lymphocytes, helper cells and other components of the immune system that facilitate activation of T lymphocytes. If such coincubation results in incorporation of tritiated thymidine into DNA it would be a clear

indication of an autologous activation of the immune system. As a control, the same buffy layer will be incubated with the non-transfected parental glial cells from the primary tumor in the presence of tritiated thymidine. A significant difference in tritium incorporation between the two cultures will indicate activation of the immune system. As a positive control nontransfected glioblastoma cells will be incubated with buffy layer from a different individual and tritiated thymidine incorporation measured. We expect in this system a significant incorporation of thymidine into DNA in the cells of the buffy layer, since this is equivalent to an allogenic reaction. This information will be correlated with the dose of cells injected, and used to predict the patient's response.

- *Data analysis*

The initial toxicity trial is descriptive in nature. The magnitude of initial response in both clinical and laboratory analyses will enable us to determine the power needed for adequate sample sizes in a larger, randomized clinical trial to follow. In an expanded study, decision whether outcome is a success or failure would be based on measurement of tumor volume by MRI and on longevity. Factors to be paired in outcome analysis will be age, sex, tumor type and stage, and the Karnofsky performance status. The data for this study will be obtained and coordinated by the investigators and a nurse assistant dedicated to this project. Records will be managed through the facilities of the Ireland Cancer Center.

2. Patient Selection (Population)

Size and Source of Study Population and Mechanism for Contacting Subjects

Patients older than 18 years of age will be recruited from the neurosurgery services of University Hospitals of Cleveland, Cleveland Veteran's Administration Hospitals and related hospitals of the University Hospitals ' group. Patients with newly diagnosed glioblastoma multiforme will be considered for experiemental treatment. Our past experience with the prevalence of glioma at these institutions suggests we would be able to recruit 50 patients per year with glioblastoma who meet the entrance criteria defined for this study.

Number to be studied and design of experiments. Twelve patients will be studied. The patients will be divided into three experimental groups consisting of four patients each. There will be four patients at each cell dosage level. This study is designed to elicit evidence of toxicity. Direct evidence of efficacy will be sought in a subsequent randomized clinical trial.

Patient No.	Dose of transfected, irradiated cells	Treatment Schedule
		Day 1 — Wk 4 — Wk 12
4	10×10^6	→ → →
4	50×10^6	→ → →
4	200×10^6	→ → →

The first cohort of four patients with tissue diagnosed glioblastoma multiforme will be treated according to the above schedule with 10×10^6 cells obtained in cell culture from self and as modified by transfection with the pAnti-IGF-I vector. Prior to subcutaneous injection, the cells will be irradiated with 5,000 cGy delivered by cobalt source. In our experience this treatment is sufficient to bring about loss of the capacity to proliferate or to incorporate ^3H thymidine into DNA in cultured rat or human cells. When non-transfected rat glioma cells are irradiated with this dose they also lose the ability to product subcutaneous tumors in syngeneic animals.

If unacceptable toxicity is not obtained in the first cohort of patients treated, the second and third cohorts of of 4 patients each would receive the increased doses of cells in sequential order. Each cohort of patients would be separated in time by an interval of three months in order to determine toxicity and side effects before proceeding to the greater dose level.

Exclusion/inclusion criteria Patients with suspected glioblastoma multiforme based on clinical presentation and radiologic evaluation will be asked to particate in this trial. Patients will be asked for informed consent and entered into the protocol prior to surgery.

Inclusion Criteria:

1) Injection of transfected cells will not take place unless the tumor histopathology fulfills the criteria of glioblastoma multiforme and the cultured tumor cells demonstrate IGF-I production, as described above.

2) Age > 18 years

3) Completed course of radiotherapy (4500 cGy to the tumor with 3 cm margins and 1500 cGy boost to tumor bed

4) Glucocorticoids reduced to the lowest stable dose possible

Exclusion Criteria:

1) Histologically verified tumor other than glioblastoma multiforme

2) Epstein/Barr virus antibody panels will be obtained on all potential study patients. Patients with panels consistent with active ongoing infection will be excluded. The reason for this exclusion is that we are introducing a vector that contains components of Epstein/Barr virus. These components in themselves will not reproduce viable virions. However, we do not want to study the possible interactions between the transfected element and ongoing infection.

3) HIV antibody positive patients will also be excluded from this study. Although the vector used for transfection in these studies consists of components of Epstein/Barr virus DNA that do not include the immediate early region, previous work has shown that the immediate early gene products of some members of the herpes virus family can transactivate HIV promoter.

4) Pregnancy testing will be performed on all eligible women. No pregnant woman will be entered into the study.

5) Coagulopathy. Patients must have a prothrombin time (PT) and activated partial thromboplastin time (PTT) no greater than 1.4 x controls

6) Thrombocytopenia. Platelet count must be >60,000/mm3.

7) Acute or active infection requiring treatment will delay or exclude patients depending on severity, type and treatment.

8) Granulocytopenia. Total neutrophil count must be greater than $800/mm^3$

9) Lymphopenia. total lymphocyte count must be greater than $500/mm^3$

10) Severe systemic disease. Lung or heart disease associated with an unacceptable anesthetic/operative risk will exclude patients from this study.

11) Patients with a Karnofsky Score below 40 (see Attachment A)

12) Patients who are unable to understand and sign the informed consent will be excluded from the study.

13) Patients who have undergone chemotherapy will be excluded from the study

14) No prior history of malignancy other than curatively treated non-melanoma of the skin or carcinoma of the cervix in-situ

3. Justification for using subjects with compromised ability to give consent

not applicable

4. Financial considerations

No compensation will be offered to research subjects or their families. The cost for the Clinical Research Center hospitalization, monitoring of patients and recording of data, and all cost for tissue culture and preparation of transfected cells will be borne by the study. Other costs to be charged to the patient's insurance would include most procedures that would be performed at this frequency in routine management. With the exception of the the MRI obtained on the day prior to treatment, the various MRI studies outlined here would be obtained during the routine followup of these patients even if they were they not in the study. Therefore this cost would not be considered as added expenses incurred by participation in the study.

IV. Risks and benefits

Risk/Benefit Ratio

Benefits: The curative effects of this therapy in rats may not be transferable to the humans because of basic differences between rat and human glial tumors and immune systems. Alternatively, patients may already be so immunosuppressed by their neoplasms that they cannot mount a response sufficient to impact the existing tumor burden. However, this therapy might be effective despite theoretical obstacles. Therefore, the major potential benefit of this study to a glioma patient will be the development of an effective immunotherapy which will result in the regression of his or her tumor. This approach to active immunization could provide lasting and sustained anti-tumor effects, in contrast to other models of immune therapy which are time-limited and require harvesting of large quantities of the patient's own immune cells (41). New knowledge gained in the area of tumor immunology and the interaction of tumor and host will provide another potential benefit. Finally, studies such as this one provide psychological benefit to patients with fatal diseases by giving them a sense of hope and of active participation in therapy, as well as by creating an altruistic outlet.

Risks and Precautions to minimize risks: One potential adverse effect could be the development of focal peri-tumoral cerebral edema. Study patients may have significant residual or recurrent tumor mass at the time of antisense treatment. If injection of transfected tumor cells provokes an immune-mediated lytic response against tumor cells, release of various mediators and associated tumor necrosis could provoke a temporary worsening of pre-existing tumor swelling or vasogenic cerebral edema. If severe, a shift in neural structures could worsen a pre-existing neurologic deficit and even lead to coma or death. Therefore, patients will be followed closely with serial neurologic examinations and MRI studies. Despite the availability of this imaging modality, it may not be possible to distinguish worsening due to growth of the tumor from that due to cerebral edema in response to changes in the tumor induced by the antisense therapy. If worsening of the neurologic status specifically due to increased focal cerebral edema develops, the patient will be treated with high-potency glucocorticoids (Decadron). If the patient is already on these agents the dose will be increased

as tolerated to a maximum of 10 mg every four hours. If the patient should continue to deteriorate, or if impending transtentorial herniation is evident, decompressive craniotomy may be offered to the patient.

As with any invasive procedure, infection is a risk. Patients with glial tumors are often immunocompromized by their tumors. In addition they may have increased susceptibility to infection because of chronic glucocorticoid administration. The injection process itself carries little risk of infection. However contamination of cells during culture, passage or transfection will be carefully avoided by extensive precautions against contamination of cultured material. Tumor cells will be harvested in the operating room at University Hospitals of Cleveland and immediately placed in culture media in a sealed sterile vial. Samples will be transported to the culture lab which is dedicated exclusively to this project. There, under a Category 2 laminar flow hood, samples will be minced and dissociated as described. Glioma cultures will be maintained without antibiotics, and will be screened regularly by culture for bacteria, mycoplasma and fungi.

Prior to injection, transfected tumor cells will be irradiated. Therefore growth of transfected cells at the site of local injection should not occur. Bloom injected untransfected human glioma cells under the skin of the same patients from whom they had been obtained (50). Ten of 12 patients developed tumor growth at the site of injection. However Mahaley's group showed that untransfected glioma cells injected with BCG caused an occasional severe local reaction, but no autoimmune encephalitis or tumor metastasis (29). Since cells in this protocol will be rendered non-viable by radiation prior to injection, this should be unlikely to occur. Because the cells to be reinjected in this study will have a major growth factor pathway suppressed by antisense IGF-I, unrestrained growth of the tumor cells is theoretically less likely. Our previous experience with the rat model showed that transfected cells never became tumors, because they are unable to produce IGF-I and in addition propagate a T cell mediated immune response within the host (30). Nevertheless as part of their follow-up, patients will be monitored for tumor growth at the site of injection. Should a tumor mass develop, its size and growth rate will be monitored on a frequent basis. If the mass does not regress, or if functional limitation or pain develops, the mass will be entirely resected, followed by local radiation therapy. Even if no tumor growth occurs at the site of injection, needle biopsy of the subcutaneous pocket will be performed 4 weeks following injection of transfected cells to assess for the presence of viable tumor, CD8+ killer T cells and other immune mediators.

Immunologic attack on the tumor and the injected transfected cells could lead to local immune-mediated phenomena such as swelling and pain at the site of injection. Clinically this might be difficult to distinguish from a bacterial or fungal infection at the injection site. Under such circumstances, blood cultures will be obtained and appropriate intravenous antibiotics will be administered. If cultures are negative, and the condition does not respond to broad spectrum antibiotics, biopsy of the injection site will be performed to establish a diagnosis. Ulceration occurred in the study in which tumor cells were injected with BCG adjuvant (29). Severe reactions are less likely in the absence of adjuvant.

Although CD8+ killer T cells are involved, the precise mechanism by which antisense transfection works in the rat model is not known. Expression of the antisense vector and subsequent downregulation of IGF-I may lead to an unmasking or increased expression of oncofetal antigens on the surface of transfected cells. These antigens might also be present in smaller amounts or in a slightly different form on untransfected tumor cells. Therefore it is possible that other antigens present on both tumor and normal glia could be affected and become susceptible to immune-mediated killing. Under such circumstances, toxicity could conceivably affect normal cells within the central nervous system, producing an allergic encephalomyelitis or peripheral neuritis. Target antigens shared by other organ systems could similarly be attacked. Such a situation, while unlikely, would be initially difficult to detect. The potentially serious effects of this scenario would be treated with immunosuppressant therapy. To date no evidence for such a phenomenon has been detected in laboratory animals in which antisense therapy has been studied or in humans who have undergone other forms of immunotherapy for glioma (29). There are however examples of allergic encephalomyelitis in animals immunized with antigens derived form autologous nervous tissue combined with Freund's adjuvant.

A final potential risk is the failure of the transfected cells to elicit any response at all, due to inadequate doses of transfected cells or local host factors. The site of subcutaneous injection in each case will be the forearm, because this site has previously been shown to result in more effective antibody formation with traditional immunization for pneumococcal and hepatitis vaccine.

Human applications of gene therapy for cancer are very few at present. This project may offer some theoretical advantages when compared to human trials of gene therapy already ongoing. First, the transfected tumor cells will be injected into a subcutaneous site which can be readily observed, and even totally resected if necessary. Second, we are using human, not animal cells, at a site distant from the brain. Repeat surgery or stereotactic placement of the transformed cells will not be required. Thirdly, the effector limb of this therapy is the tumor patient's own immune system. No exogenous medications such as IL-2 or gangciclovir will be required to produce the desired therapeutic effect in the transfected cells. Therefore the drug toxicity associated with such agents is eliminated.

Monitoring of Toxicity/Reporting of Adverse Reactions/Stop Criteria:

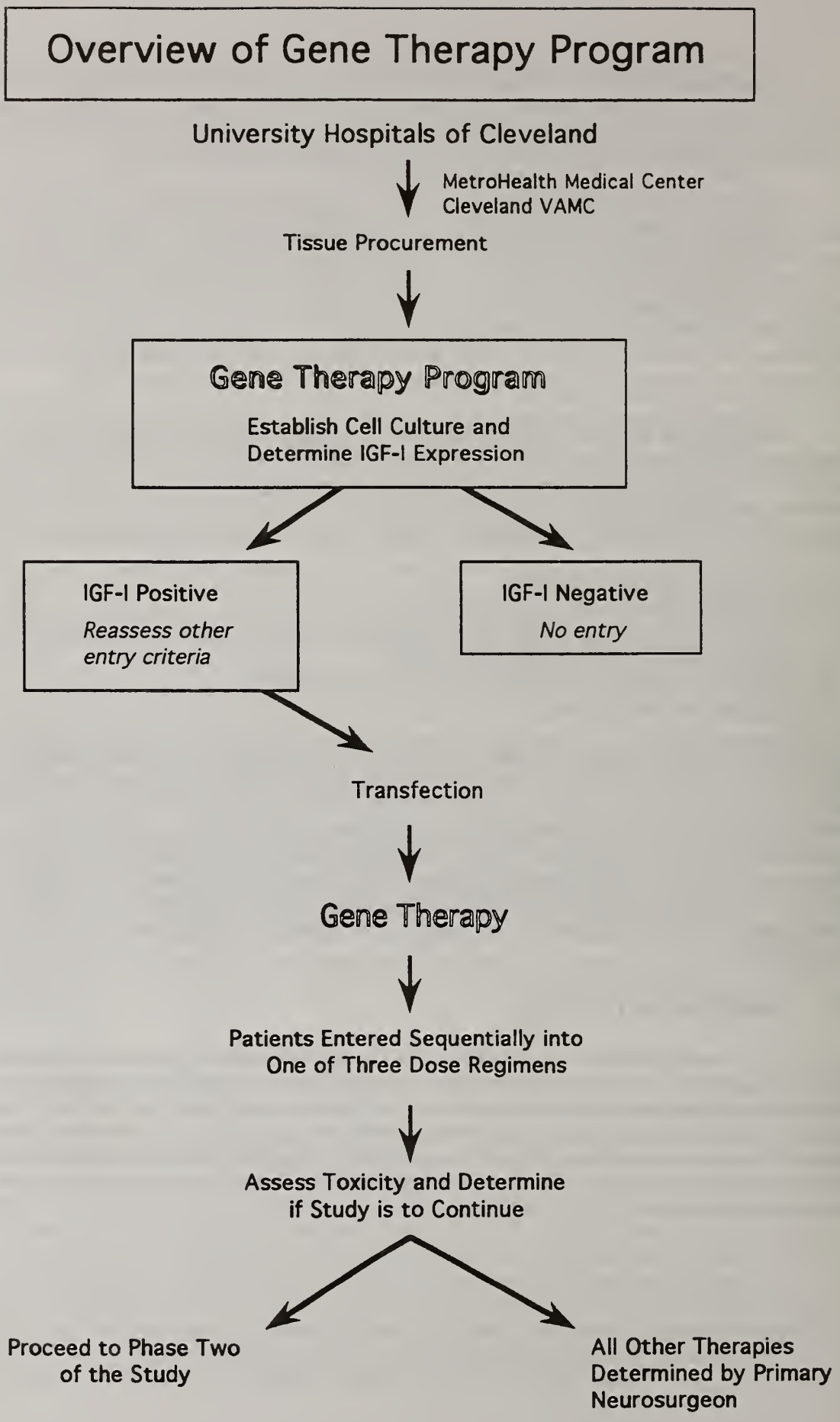
Potential complications and toxicities and approaches to their control were described in the previous section. Any significant deterioration in mental status or neurological function, or evidence for altered immunity and/or infection that is not readily attributable to the underlying disease process will be cause to interrupt the study for all subsequent patients. Examples of such changes would include:

1. Infection at the site of injection
2. growth of tumor at the site of injection
3. deterioration in neurologic function not attributed to normal progression of tumor growth or to other metabolic or systemic derangements
4. an immune reaction not attributable to drugs or medications
5. sudden unexplained death

The adverse reactions would be reported promptly to the Investigational Drug Branch (IDB), phone (301) 496-7957. ADR reports will be made even if there is only a suspicion of an adverse effect. Life-threatening reactions and/or deaths while on treatment will be reported to the NCI by phone within 24 hours. A written report will follow within 10 working days. Written reports will be sent to Investigational Drug Branch Cancer Therapy Evaluation Program P.O. Box 30012 Bethesda, MD 20824. The course of the study would remain interrupted until a review of the circumstances involved has been made by the primary investigators and the Gene Therapy Subcommittee of the Ireland Cancer Center at Case-Western Reserve University, and until appropriate solutions to the problem have been determined.

V. Justification of Study

The success in treatment of glioma has not increased significantly over the past several decades. The only success achieved is a modest increase in longevity and this is dependent upon radical excision of early and anatomically accessible lesions. The overall survival remains dismal and the disease is almost uniformly fatal. These statistics warrant an aggressive search for new therapies. The data presented here in studies using an anti-sense IGF-I strategy in treatment of rat glioblastoma represent a unique approach to the induction of the immune system. The results merit a trial of the same strategy in human subjects.


Overview of Gene Therapy Program
University Hospitals of Cleveland
MetroHealth Medical Center
Cleveland VAMC
Tissue Procurement
Gene Therapy Program
Establish Cell Culture and
Determine IGF-I Expression
IGF-I Positive
Reassess other
entry criteria
IGF-I Negative
No entry
Transfection
Gene Therapy
Patients Entered Sequentially into
One of Three Dose Regimens
Assess Toxicity and Determine
if Study is to Continue
Proceed to Phase Two
of the Study
All Other Therapies
Determined by Primary
Neurosurgeon

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45

UNIVERSITY HOSPITALS OF CLEVELAND
PATIENT CONSENT FOR INVESTIGATIONAL STUDIES

TITLE OF PROJECT:

Attachment G

Effect of Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I on Tumorigenicity of Human Brain Tumor

DESCRIPTION OF STUDIES:

I am being invited to take part in a research study that concerns the treatment of malignant brain cancers. My participation in the study is entirely voluntary. I am aware that I may not benefit directly from taking part in the study. However knowledge may be gained that will benefit future patients with brain tumor. At any time I may withdraw from the study without changing my usual health care and without penalty or loss of benefits to which I might otherwise be entitled. The nature of the study, the risks, inconveniences, discomforts and other information have been discussed with me. I have been encouraged to discuss any questions I might have about this study with the staff members who explained it to me.

There is currently no cure for malignant brain tumors which have been classified as glioblastoma multiforme. Treatment including surgery, radiation therapy and/or chemotherapy cannot prevent recurrence of tumor. However, if I choose not to participate in this study my doctors will make available and discuss with me alternative forms of treatment including surgery and radiation or surgery alone, chemotherapy or no treatment at all.

A new experimental approach for treatment of these tumors that takes advantage of knowledge gained in gene therapy research has been developed. An excessive production of a protein,an "insulin like growth factor" called IGF-1, has been found in a number of different cancers. These include brain cancers such as the one I have. Why the cancer makes an excessive amount of this growth factor and what its specific role is in the cancer process are not yet fully known.

The understanding of molecular biology has been used to design a vector which would change how cancer cells make this growth factor. A vector is in ways similar to a taxi cab, a vehicle which transports passengers to specific sites. In this case the vehicle is used to transport new genetic information into living cells. Genetic information is carried within the deoxyribonucleic acid (DNA) of cells or viruses. The DNA is the master computer which directs and controls the synthesis of molecules (RNA), that normally act as templates for production of proteins and other components essential to cell or virus structure and function. The taxi is itself composed of a very small portion of virus DNA that has been genetically altered to make it harmless to humans. The passenger is DNA that directs the synthesis of RNA molecules (antisense RNA) that are not normally produced in the cell and that interefere with the usual RNA needed to produce the growth factor, IGF-I. When this happens, the cell stops being able to make new IGF-I. This vehicle and its passenger have been introduced into glioblastoma

CONSENT FORM **ATTACHMENT G**

cancer cells in animals, and these cells have been injected back into the animals. When this is done the genetically altered cells gradually disappear and the primary cancer resolves.

If samples of the original cancer tissue from the animal with cancer are looked at as the cancer is disappearing, a strong immune reaction is found. Thus the animal's body is actively rejecting the tumor. The main cells which appear to be causing this rejection are called T lymphocytes. The T lymphocytes attack and destroy the glioblastoma cancer cells with the anti-sense vectors in them, and also attack and destroy the main glioblastoma tumor. If a different, unrelated, tumor is also present in the same animal, the T lymphocytes do not attack it. Why the animal's immune system is turned on in a strong fashion to attack and destroy the cancer cells is not known.

Although these results are very encouraging, it is not at all certain that the studies in animals will translate into treatments in human beings. The purpose of this study is to gain information about whether or not this new approach will be effective in treating human brain tumors. I will be one of the first people to receive this experimental treatment for brain tumors. While it is hoped that information on the usefulness of this approach in humans, will be gained, it is not known if my tumor will get smaller as a result of this treatment.

The usual treatment for my brain cancer, which prescribed by my personal Neurosurgeon, will be exactly the same whether or not I participate in this study. The additional research procedures are described next.

At the time of surgery done to remove as much of the brain cancer as possible, or at the time of biopsy to confirm the characteristics of my tumor, a small sample of cancer cells (less than the size of a pencil eraser) will be obtained and these cells will be examined by the pathologist and will be cultured under sterile conditions in the laboratory. If my tumor is a glioblastoma multiforme, and expresses IGF-I, I will be eligible to continue in the study. An additional requirement that I agree to follow before entry into this study is that I will use contraception measures advised by my doctor, and if I am of child-bearing age and female, I will obtain a pregnancy test to be certain that I am not pregnant before any treatment is begun.

At a predetermined time an attempt would then be made to alter the genetic material of my brain tumor cells growing in the laboratory. A vector which carries the gene that produces anti-sense RNA to insulin-like growth factor-I

(IGF-I) would be inserted into the growing tumor cells. The vector consists of parts of a virus which can divide and make multiple copies of itself once it is inside of the cancer cells. The virus has been made inactive as an agent which can cause infection by taking away parts, so it cannot make whole new viral particles inside the cell. However the vector will make multiple copies of the anti-sense RNA that blocks the formation of insulin-like growth factor-I.

What will my participation in these experiments involve?

Once my brain tumor cells have been genetically altered in the laboratory, they will be injected back under the skin of one of my arms using a needle and syringe. Like any immunization or "shot" this procedure will be done while I am awake. An injection under the skin is a simple procedure usually done on outpatients. However, since the injection of genetically altered tumor cells into humans has not been done before, I will be hospitalized for close observation. All told, I will receive a total of 3 or 4 injections spaced 4 weeks apart.

I will be kept in the hospital for 24 hours to check for an allergic reaction, such as local redness, or if more severe, flushing of the skin or wheezing. My doctors do not expect such a reaction since I will be receiving my own cells back. However, if I should develop such a reaction I would receive medications such as antihistamines while in the hospital. Another possible complication of injecting back my tumor cells could be introducing an infection under my skin. This complication is unlikely because the cells are grown under sterile conditions and tested to insure that they are free of bacteria or virus before they are injected back under my skin. However I will be monitored closely for development of infection by checking my temperature and by obtaining complete blood counts three times per week during the first week and once a week thereafter. Should an infection occur, appropriate antibiotic therapy will be started.

I will need to come back to see the research doctors three times in the first week for physical and neurologic examination and blood testing. I will come back weekly for four weeks, and every two weeks for four months, then monthly thereafter for at least one year. I will continue to be followed at less frequent intervals for life. My doctors will check me closely to make sure I have not developed any complication. They will examine my arm for signs of infection or evidence of a reaction. A skin biopsy of the injection site will be made three months after the genetically altered tumor cells are injected under my skin.

CONSENT FORM

p 4. Effect of Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I on Tumorigenicity of Human

Blood for special tests will be drawn at the start of the study and at each one of my clinic visits. Approximately 2 tablespoon of blood will be drawn at each visit. I will receive skin tests (like a tine test for tuberculosis,Tb) at the start of the study and at six months. I will have additional MRI scans of the head performed 4, 8 and 16 weeks, after the cells are injected.

Because this gene therapy is a new form of treatment, all risks cannot be foreseen or eliminated. It is even possible that I may experience a life threatening complication as a result of my participation in this study. In the event of my death even if it does not arise because of the cancer, an autopsy will be requested. All costs related to culturing my cancer cells in the laboratory in order to expand the numbers of cells, for laboratory studies needed to rule out infection in the cultured cells, for altering these cells genetically, and for monitoring my health during the 48 hour period following the injections, will be covered by research funds. I will be followed for routine care by my personally selected neurosurgeon and physician. Costs for any complications arising from this study and for follow up visits specifically related to the research study will be born by the study program.

I have read the preceding consent form information and understand that this treatment which I will receive is part of a clinical research study. I know that it is not possible to predict if I will receive personal benefit from participation in this study and that there may be certain unpredicted risks associated with this treatment. I have the option of not participating in this study. If I do participate I may withdraw at any time. Any additional care I should choose to have at this institution would not be jeopardized by such action.

I understand that a record of my progress while on the study will be kept in confidential form at University Hospitals of Cleveland, and that the confidentiality of these records will be carefully guarded. These records may be reviewed in a confidential manner by outside agencies such as the National Institutes of Health, the National Cancer Institute, the Federal Food and Drug Administration, the manufacturer of the vector or by persons authorized to review this research study at Case Western Reserve University. No information by which I can be personally identified will be used or published. New findings from this study will be made known to me.

CONSENT FORM

p 5. Effect of Episome -Based Antisense cDNA Transcription of Insulin-Like Growth Factor-1 on Tumorigenicity of Human Brain Tumors

Dr. Fenstermacher or Anthony has described to me what is going to be done, how it is going to be done and the risks, hazards and benefits involved, and will be available for questions at (216) 791-3800 ext. 5407 or 368-3388.

I understand that my decision to participate or not to participate in this study will not alter my usual health care. In the use of information generated from this study, my identity will remain anonymous. I am aware that I may withdraw from this study at any time. I further understand that in the event of physical injury or illness occurring to me resulting from the research procedures, University Hospitals will not provide free medical care or compensation for lost wages. Further information with respect to this topic is available from the Office of the Chief of Staff. I understand that by signing this consent form, I do not waive any of my legal rights nor does it relieve investigators or suppliers of liability, but merely indicates that I have been informed about the research study in which I am agreeing to participate. A copy of this form is available to me upon request.

Signature_____ Age_____
Date_____

Parent or Guardian Signature (if subject is minor)_____

Witnessed by_____ Date_____
(Signature of Project Investigator)

IRB
(4/89)

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Ilan, Joseph	Professor

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
The Hebrew University, Jerusalem	B.Sc.	1960	Biology
The Hebrew University, Jerusalem	M.Sc.	1962	Biology
McGill University, Montreal-Quebec,Canada	Ph.D.	1965	Biochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professor, Case Western Reserve University, Institute of Pathology, 1992-Present.
Professor, Case Western Reserve University, Dept. of Developmental Genetics & Anatomy, 1976-1992.
Professor of General Medical Science (Oncology) Case Western Reserve University, School of Medicine, (Secondary Appointment) 1987-1992.
Associate Professor, Case Western Reserve University, Dept. of Developmental Genetics & Anatomy, 1971-1976, Tenure 7/1/71
Associate Professor, Temple University, Dept. of Biology, 1970-1971, Tenure 7/1/70
Assistant Professor, Temple University, Dept. of Biology, 1966-1970
Research Associate, Rockefeller University, in collaboration with Dr. Fritz Lipmann, 1964-1966.

Selected Publications (From a total of 80)

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CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

VIROGENETICS

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January 8, 1993

Dr. Nelson Wivel, Director
Office of Recombinant DNA Activities
National Institutes of Health
Building 31, Room 4B11
Bethesda MD 20892

Dear Dr. Wivel:

This letter is to formally initiate a request for reduction in required Biosafety Level for three of our poxvirus vectors, NYVAC, ALVAC, and TROVAC. We wish to present our proposal to the RAC at the March 2, 1993 meeting.

The Office of Recombinant DNA Activities (ORDA) recognizes that several different combinations of laboratory practices, containment equipment, and laboratory design may be appropriate for containment of specific research activities. The selection of alternative methods of primary containment is dependent on the level of biological containment provided by the host-vector system (*Guidelines for Research Involving Recombinant DNA Molecules, Appendix G-II*). We are asking for a reduction in containment level for three of our recombinant poxviruses, NYVAC, ALVAC and TROVAC from BL-2 to BL-1 because we believe they "do not present a significant risk to health or environment" (*Ibid, Section IV-C*), due to the extent of biological containment provided by their construction. Such a change in classification would more accurately reflect the pathogenicity of the organisms and their potential hazard to man, as well as facilitate the development of safe and efficacious human and veterinary vaccines based on these vectors.

According to the *Guidelines,* any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized:

i) survival of the vector in its host outside the laboratory
ii) transmission of the vector from the host to other non-laboratory hosts

We will demonstrate minimization of these types of "escape" for both vectors, via biological containment, to a degree which we believe justifies the reduction in biosafety level we are requesting.

VIROGENETICS

Enclosed are three copies, as requested, of background information for each vector. We have prepared the information for each vector in separate packages in order to reduce the size of the package and to maintain the distinctions between the vectors. Dr. Enzo Paoletti will present the proposal to the committee. He will use either a standard 2 X 2 slide projector, or an overhead projector, whichever is preferable.

If you have any questions or comments, please do not hesitate to contact me at extension 404.

Sincerely,

Arvilla L. Trag
Regulatory Affairs/
Quality Assurance Supervisor

cc: Kent Van Kampen, CEO
Enzo Paoletti, Director of Research
James Tartaglia, Assistant Director of Research
file

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TROVAC

REQUEST FOR REDUCTION IN BIOSAFETY LEVEL REQUIREMENTS FOR THREE RECOMBINANT POXVIRUS VACCINE VECTORS-- NYVAC, ALVAC, AND TROVAC

The Office of Recombinant DNA Activities (ORDA) recognizes that several different combinations of laboratory practices, containment equipment, and laboratory design may be appropriate for containment of specific research activities. The selection of alternative methods of primary containment is dependent on the level of biological containment provided by the host-vector system (*Guidelines for Research Involving Recombinant DNA Molecules, Appendix G-II*). We are asking for a reduction in containment level for three of our recombinant poxviruses, NYVAC, ALVAC and TROVAC from BL-2 to BL-1 because we believe they "do not present a significant risk to health or environment" (*Ibid, Section IV-C*), due to the extent of biological containment provided by their construction. Such a change in classification would more accurately reflect the pathogenicity of the organisms and their potential hazard to man, as well as facilitate the development of safe and efficacious human and veterinary vaccines based on these vectors.

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ii) transmission of the vector from the host to other non-laboratory hosts

We will demonstrate minimization of these types of "escape" for both vectors, via biological containment, to a degree which we believe justifies the reduction in biosafety level we are requesting.

The format in which we are presenting our information is similar to that used by the USDA for their Veterinary Biologics Summary Information Format. Briefly, the format provides a synopsis of specific characteristics of the recombinant, with attached appendices for further detail. Each recombinant will be described and appendicized separately. For clarity, a glossary is enclosed on the following page.

VIROGENETICS

TROVAC (FPpp) INFORMATION

A. ORIGIN OF THE TROVAC (FPpp) VECTOR

1. **Parental organism:** The parental organism FP-1 is an attenuated vaccine strain of fowlpox virus originally isolated from a pox scab on a chicken in France. The virus was attenuated by approximately 50 serial passages in chicken embryonated eggs followed by 25 passages on chicken embryo fibroblast cells. In September 1989, a master seed virus DCEP25 was established by Rhone Merieux, Lyon, France. The virus was imported to the U.S. by Select Laboratories, Inc. under permit #20374. The virus is used by Rhone Merieux to produce a live fowlpox vaccine registered in France as "DIFTOSEC CT".

2. **Description of vector:** The fowlpox virus FP-1 was sent to Virogenetics from Select Laboratories, Inc. under USDA permit #21671 in September 1989. After four successive plaque purifications, one plaque isolate was amplified in primary CEFs to establish a seed stock virus designated as TROVAC (FPpp). No other genetic modifications were used to produce the TROVAC (FPpp) vector.

B. BIOLOGICAL PROPERTIES

1. **Host/range specificity:** Productive replication of avipoxvirus is naturally restricted to avian species (Esposito, 1991 and Cox *et al.*, 1991). This characteristic has been preserved in TROVAC (FPpp) and recombinants based on TROVAC (FPpp) (Appendices A, B, and C). *In vitro* it grows in primary and secondary CEFs and chick dermal cells, but not in mammalian cells (Appendix A). Attempts were made to adapt FP-1 or the recombinant vFP3 to grow in non-avian cells by a series of six sequential blind passages on Vero and MRC-5 cells without success (Appendices B & C).

   Fowlpox virus has been used as a live attenuated vaccine since the 1920s to control the disease in poultry (Appendix D). In the history of the virus as a vaccine in the poultry industry, there are no reports of transmission of the virus from vaccinated birds to humans or any other species (Appendices C & E).

2. **Virulence:** Commercially available fowlpox vaccines in the U.S are either of chick embryo origin and contain live, nonattenuated fowl poxvirus, or are attenuated vaccines of cell culture origin (Tripathy, 1991). In a study using a TROVAC-based recombinant with the HA gene of a virulent strain of avian influenza virus incorporated into the vector's genome (vFP11), non-vaccinated contact control birds were housed with vaccinates, and no evidence of spread to non-vaccinated birds was shown. These contact control birds did not survive challenge (Appendix F).

   In a study done on swine to observe local reactions after intradermal inoculation with four recombinants, including FP-1 and FP-RG (vFP6), reactions to both inoculants was very low, even at 10^8 $TCID_{50}$, with FP-RG (vFP6) appearing to be even less reactive than the parental strain FP-1 (Appendix G).

   When FP-RG (vFP6) was inoculated into chick embryos, there was no effect on hatchability or on the health of the hatchlings. One-day-old chickens and 28 day-old chickens inoculated by the IM route remained completely healthy during the 28 day observation period, with no sign of reactivity at the site of inoculation. Twenty-eight-day-old birds inoculated via the cutaneous route (wing web) showed very mild reaction at the site of inoculation, which cleared completely by the end of the observation period (Appendix H).

3. **Immunogenicity of fowlpox-based vectors:** The fowlpox-based vaccine against avian influenza (vFP11), when administered to chickens via wing-web route, gave complete protection from challenge after a single inoculation (Appendix F). Fowlpox-based vaccines administered to chickens against Newcastle disease virus gave complete protection from challenge after single IM or wing-web inoculations (Appendices I and J). Despite the restriction of productive replication in non-avian species, fowlpox-based recombinants are capable of expressing extrinsic immunogens and eliciting humoral and cellular immune response in mice, rabbits, cats, dogs, cattle and swine (Appendices A, B and G).

4. **Environmental distribution:** It is anticipated that the TROVAC (FPpp) strain of fowlpox virus will have the same range of environmental distribution as the currently used commercial vaccine strains. Due to the avian-restricted host-range of TROVAC (FPpp), no vertical transmission from avian to mammal or mammal to avian is anticipated.

5. **Geographical distribution:** The TROVAC (FPpp) vector has the potential to be used as a vaccine for a wide variety of veterinary diseases world-wide. The highly attenuated phenotype of the TROVAC (FPpp) vector coupled to its potency as an immunization vehicle provides a favorable benefit-to-risk ratio. It possesses the physical characteristics desirable in a live vaccine candidate, i.e. genetic and environmental stability, yet has attenuation characteristics consistent with an enhanced safety profile.

6. **Recommended NIH/CDC Biosafety Level:** The NIH/CDC recommended Biosafety Level for avipoxviruses is Biosafety Level 2. Avian pox is not of public health significance (Tripathy, 1991). The USDA has assigned BL1 and BL1-LS to our TROVAC-based NDV vaccine, vFP96, based upon their risk assessment of the current available information on the recombinant (Appendix K). Due to the attenuated nature of TROVAC (FPpp), the inherent host-range restriction of avipox viruses, and the safety data presented for both TROVAC (FPpp) and TROVAC-based recombinants, we are requesting reduction to Biosafety Level 1.

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TAB	SUMMARY OF TROVAC APPENDICES
A	"Fowlpox virus based recombinant vaccines", Taylor, *et al., Technological Advances in Vaccine Development,* (ed. Laurence Lasky) Alan R. Liss, Inc., NY, 1988, pp 321-334.
B	"Recombinant fowlpox virus inducing protective immunity in non-avian species", Taylor, *et al., Vaccine*, 6:497-503, 1988.
C	"The use of fowlpox virus vectors to vaccinate non-avian species", Taylor, *et al., The Release of Genetically-engineered Micro-organisms*, (eds. M. Sussman, C.H. Collins, F.A. Skinner, D.E. Stewart-Tull) Academic Press, San Diego, CA, 1988, pp 89-103.
D	"Immunity to fowlpox", Tripathy, *et al., American Journal of Veterinary Research*, 36:541-544, 1975.
E	"Fowlpox virus as a vector in non-avian species", Taylor, *et al., Vaccine*, 6:466-468, 1988 (review).
F	"Protective immunity against avian influenza induced by a fowlpox virus recombinant", Taylor, *et al., Vaccine*, 6:504-508, 1988.
G	A report on an experiment to compare swine reactions following intradermal inoculations of both parental fowlpox and recombinants, showing no clinically significant reactivity (Rhone Merieux report #BL/AR-DRV 499.88).
H	Results of a safety study done in chicken embryos, 1-day hatchlings, and 28-day chicks, showing TROVAC-RG (vFP6) to have no abnormal effect on any of the three paradigms (Rhone Merieux report #JFB/AR-DRV 411.88).
I	"Newcastle disease virus fusion protein expressed in a fowlpox virus recombinant confers protection in chickens", Taylor, *et al., J. Virology,* 64:1441-1450, 1990.
J	"Protection of chickens with a recombinant fowlpox virus expressing the Newcastle disease virus hemagglutinin-neuraminidase gene", Edbauer, *et al., Virology*, 179:901-904, 1990.
K	Letter from Dr. Joseph Chen of USDA/APHIS, assigning BL1 containment level to the fowlpox recombinant NDV vaccine, vFP96.

VIROGENETICS

ALVAC

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REQUEST FOR REDUCTION IN BIOSAFETY LEVEL REQUIREMENTS FOR THREE RECOMBINANT POXVIRUS VACCINE VECTORS-- NYVAC, ALVAC, AND TROVAC

The Office of Recombinant DNA Activities (ORDA) recognizes that several different combinations of laboratory practices, containment equipment, and laboratory design may be appropriate for containment of specific research activities. The selection of alternative methods of primary containment is dependent on the level of biological containment provided by the host-vector system (*Guidelines for Research Involving Recombinant DNA Molecules*, *Appendix G-II*). We are asking for a reduction in containment level for three of our recombinant poxviruses, NYVAC, ALVAC and TROVAC from BL-2 to BL-1 because we believe they "do not present a significant risk to health or environment" (*Ibid, Section IV-C*), due to the extent of biological containment provided by their construction. Such a change in classification would more accurately reflect the pathogenicity of the organisms and their potential hazard to man, as well as facilitate the development of safe and efficacious human and veterinary vaccines based on these vectors.

According to the *Guidelines,* any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized:

i) survival of the vector in its host outside the laboratory

ii) transmission of the vector from the host to other non-laboratory hosts

We will demonstrate minimization of these types of "escape" for both vectors, via biological containment, to a degree which we believe justifies the reduction in biosafety level we are requesting.

The format in which we are presenting our information is similar to that used by the USDA for their Veterinary Biologics Summary Information Format. Briefly, the format provides a synopsis of specific characteristics of the recombinant, with attached appendices for further detail. Each recombinant will be described and appendicized separately. For clarity, a glossary is enclosed on the following page.

IROGENETICS

ALVAC (CPpp) INFORMATION

A. ORIGIN OF THE ALVAC VECTOR

1. **Parental organism:** The parent strain of canarypox (Rentschler strain) was isolated in Germany in 1970, and obtained by Institut Merieux in 1973. The virus was attenuated by 200 serial passages on chick embryo fibroblasts (CEFs). The attenuated virus was registered as a vaccine in France in 1975 under the name KANAPOX (ND). Authorization was given to market the vaccine in France in 1982 and this was renewed in 1987 under the reference number 682827.2 01.82 V. The product is also licensed in Taiwan.

2. **Description of vector:** The vaccine strain was sent to Select Labs from Institut Merieux in the fall of 1989, and then to Virogenetics. It was subjected to four successive plaque purifications under agar and one clone was amplified through five additional passages. It was designated CPpp--CanaryPox plaque purified, and is now referred to as ALVAC. No other genetic modifications were used to produce the vector.

B. BIOLOGICAL PROPERTIES

1. **Host/range specificity:** Productive replication of avipoxvirus is naturally restricted to avian species (*Recombinant Poxviruses*, p126, M. Binns ed.). This characteristic has been preserved in ALVAC (CPpp) and recombinants based on ALVAC (Appendices A, B, C, and D). *In vitro* it grows in CEFs and QT35 cells, but not in mammalian cells (Appendices A, B and C). Post-inoculation attempts to recover virus from the inoculation site (ID, IM, orally, and ocularly in squirrel monkeys) yielded virus only on days 2 and 4 post-inoculation, and only from ID sites at the higher dosage (Appendix E). Amplification of viral sequences by PCR analysis at the site of intradermal inoculation indicated only sporadic ability to detect the presence of virus beyond eleven days (Appendix E). Therefore, survival of the vector in its host outside the laboratory is not an issue.

2. **Virulence:** ALVAC (CPpp) has the attributes of an attenuated canarypox virus. *In vivo*, it does establish infection in canaries upon skin inoculation, but causes no disease or death (Appendix F). In an IC challenge of newborn mice, ALVAC (CPpp) had an LD_{50} value of 1.00×10^7 pfu; in 3-week-old mice, the LD_{50} was 1.58×10^8 pfu (Appendix G).

3. **Immunogenicity of ALVAC-based vectors:** Despite the restriction of productive replication in non-avian species, ALVAC-based recombinants are

capable of expressing extrinsic immunogens and eliciting humoral and cellular immune response in mice, cats, dogs and swine (Appendices H, I, J, & K). In four- to six-week-old mice, ALVAC-RG (vCP65) had a PD_{50} value of 3.86 $TCID_{50}$ (Appendix G). Immunization with ALVAC (CPpp) or other ALVAC-based recombinants does not preclude immune responses to novel antigens expressed by the ALVAC vector (Appendix L).

4. **Environmental distribution:** It is anticipated that the ALVAC (CPpp) strain of canarypox virus will have the same range of environmental distribution as the Rentschler vaccine strain from which it was derived. Due to the avian-restricted host-range of ALVAC (CPpp), no horizontal transmission from mammal to mammal, or vertical transmission from mammal to avian is anticipated.

   ALVAC-RG (vCP65), a recombinant rabies vaccine expressing the rabies glycoprotein G, is in Phase 1 human clinical trial at Johns Hopkins (Appendix M) and in France (Appendix N). The vaccine was assessed as being safe without any instances of significant systemic illness, significant adverse reactions at the injection site, significant classic laboratory abnormalities, or death in any of the human volunteers.

   ALVAC-gp160 MN (vCP125), an HIV-1 vaccine, and ALVAC-MV (vCP82), a measles vaccine, are both in Phase 1 human clinical trials in France.

   The USDA/APHIS has assigned Biosafety Level-1 to a contained release involving an ALVAC-based Japanese Encephalitis Virus vaccine (vCP107) to be administered to 150 swine at Purdue University (Appendix O).

5. **Geographical distribution:** The ALVAC (CPpp) vector has the potential to be used as a vaccine for a wide variety of both human and veterinary diseases world-wide. The highly attenuated phenotype of the ALVAC vector coupled to its potency as an immunization vehicle provides a favorable benefit-to-risk ratio. It possesses the advantages of vaccinia-based vaccine candidates, yet has attenuation characteristics consistent with an enhanced safety profile.

6. **Recommended NIH/CDC Biosafety Level:** The NIH/CDC recommended Biosafety Level for avipoxviruses is Biosafety Level 2. Due to the attenuated nature of ALVAC (CPpp), the demonstration of non-replication in mammalian species, the safety data presented for both ALVAC (CPpp) and ALVAC-based recombinants, and the approval of Phase I human clinical trials of ALVAC-RG (vCP65), ALVAC-MV (vCP82), and ALVAC-gp160 MN (vCP125), we are requesting reduction to Biosafety Level 1.

TAB	SUMMARY OF ALVAC APPENDICES
A	Report demonstrating the host-range restricted nature of ALVAC-RG (vCP65) in human cell lines in culture.
B	Comparison of the abilities of ALVAC (CPpp) and ALVAC-RG (vCP65) to adapt to growth in two mammalian cell lines (Virogenetics report #7-06-RAB).
C	Comparison of yield of ALVAC (CPpp) grown in avian and non-avian cells at 31°C, 37°C, and 39°C, Showing that ALVAC (CPpp) is unable to replicate in non-avian cells regardless of the temperature of incubation.
D	Report illustrating the host-range restricted nature of ALVAC-RG (vCP65) by comparing viral replication in mice and canaries.
E	Recovery of virus from inoculation site--two parts: 1) a safety study in which squirrel monkeys were inoculated with ALVAC (CPpp) and ALVAC-RG (vCP65) via four different routes and sites were swabbed for recovery of inoculated virus. 2) a study of mice in which analysis of viral recovery from site of inoculation was performed by titration of QT35 cells and by amplification of viral sequences by PCR analysis.
F	Comparison of the virulence of canarypox virus (Rentschler strain) and of ALVAC-RG (vCP65) in canary birds.
G	"NYVAC: A Highly Attenuated Strain of Vaccinia Virus", Tartaglia *et al.*, *Virology*, 188:217-232 (1992). This paper presents safety data on ALVAC (CPpp) and ALVAC-RG (vCP65) and compares them to various strains of vaccinia and vaccinia-based constructs.
H	"Highly attenuated Poxvirus Vectors in AIDS Vaccine Development", Cox *et al.* Submitted for publication. This paper presents data showing the ability of ALVAC-based constructs to express extrinsic immunogens and elicit protective immune responses.
I	"Efficacy studies on a canarypox-rabies recombinant virus", Taylor *et al.*, *Vaccine*, 9:190-193 (1991). This paper presents data of an efficacy study done on dogs, cats and mice.
J	"Protection of Cats Against Feline Leukemia Virus by Vaccination with a Canarypox Virus Recombinant, ALVAC-FL", Tartaglia *et al.*, manuscript submitted to *J. Virology*.

K	"Nonreplicating Viral Vectors as Potential Vaccines: Recombinant Canarypox Virus Expressing Measles Virus Fusion (F) and Heamgglutinin (HA) Glycoproteins", Taylor *et al.*, *Virology*, 187:321-328 (1992). This paper presents data demonstrating protection induced in dogs against Canine Distemper Virus (CDV) challenge after inoculation with an ALVAC-based recombinant.
L	Report demonstrating that pre-existing immunity to canarypox virus does not preclude immune responses to novel antigens expressed by ALVAC-based vaccines.
M	Two parts: 1) "Human safety and immunogenicity of a canarypox-rabies glycoprotein recombinant vaccine: an alternative pox virus vector system", Fries, *et al.*, submitted for publication. 2) Evaluation of safety and immunogenicity of ascending doses of ALVAC-RG (vCP65) in healthy adult human volunteers. Study performed at Johns Hopkins Center for Immunization Research.
N	"Immunization with canarypox virus expressing the rabies glycoprotein", Cadoz, *et al.*, *Lancet*, 339:1429-1432 (1992). This paper documents a clinical trial involving twenty-five human volunteers in which ALVAC-RG (vCP65) was found to be safe and well-tolerated. Original data is appended to the article. This study was performed at Centre Hospitalier Régionale et Universitaire de Reims, France.
O	A letter from Dr. Cyril Gay of USDA/APHIS, granting Virogenetics a Biosafety Level-1 for a contained release involving an ALVAC-based Japanese Encephalitis Virus vaccine (vCP107) to be administered to 150 swine at Purdue University.
OTHER REFERENCES	*Recombinant Poxviruses*, M. Binns and G. Smith, editors; CRC Press, Boca Raton, Florida, 1992.

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REQUEST FOR REDUCTION IN BIOSAFETY LEVEL REQUIREMENTS FOR THREE RECOMBINANT POXVIRUS VACCINE VECTORS-- NYVAC, ALVAC, AND TROVAC

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VIROGENETICS

NYVAC (vP866) INFORMATION

A. ORIGIN OF THE NYVAC (vP866) VECTOR

1. **Parental organism:** A plaque-cloned isolate of the Copenhagen vaccinia vaccine strain (VC-2) was used as the parent virus in the derivation of NYVAC (vP866). The complete DNA sequence of the VC-2 isolate is known and described in Appendix A.

2. **Description of the NYVAC vector:** The highly attenuated vaccinia virus strain, NYVAC (vP866) was derived from a plaque-cloned isolate of the Copenhagen vaccine strain by the precise deletion of 18 open reading frames (ORFs) from the viral genome. Among the ORFs deleted from NYVAC (vP866) are two genes involved in nucleotide metabolism, the thymidine kinase (ORF J2R) and the large subunit of the ribonucleotide reductase (ORF I4L); the gene encoding the viral hemagglutinin (ORF A56R); the remnant (ORF A26L) of a highly expressed gene responsible for the formation of A-type inclusion bodies; the disrupted gene (ORFs B13R/B14R) normally encoding a serine protease inhibitor; and a block of twelve ORFs bounded by two known viral host range regulatory functions (ORFs C7L through K1L). Within this block a secretory protein (ORF N1L) implicated in viral virulence and a functional complement 4b binding protein (ORF C3L) are encoded. The ORFs were deleted in a manner which prevents the synthesis of undesirable novel gene products (Appendix B). The net result of these deletions is a highly attenuated vaccinia virus which nonetheless retains the capacity to evoke strong immune responses to foreign immunogens (Appendix C).

B. BIOLOGICAL PROPERTIES

1. **Host/range specificity:** Both the C7L and K1L host range genes are deleted from NYVAC (vP866), making it deficient for replication on human cell lines (Appendices B and D), as well as cells derived from a number of other species (Appendix E). In a study comparing the replicative ability of three vaccinia strains in mouse ovaries, the WR (L-variant) and VC-2 (Copenhagen) strains disseminated to and accumulated in various organs, while the NYVAC (vP866) strain cleared rapidly and did not disseminate (Appendix F).

2. **Virulence:** In an IC challenge of newborn mice, NYVAC (vP866) had an LD_{50} value of 1.58×10^6 pfu; in 3-week-old mice the LD_{50} was 1.58×10^8 pfu. IP inoculation of high doses of NYVAC (5×10^8 pfu) into nude mice caused no deaths, no lesions, and no apparent disease over a 100-day observation

period, whereas mice inoculated with VC-2 displayed disseminated lesions typical of poxviruses (Appendix B). In rabbits inoculated with NYVAC-RG (vP879) intracerebrally, no clinical symptoms were observed over ten days post-inoculation (Appendix G). At Cornell, a total of twelve horses have been vaccinated with NYVAC recombinants, and no reactions have been observed in any horse after either primary or secondary inoculation (Appendix H). In a study involving 10 vaccinated piglets, no local or systemic reactions were observed (Appendix I). In a study of twenty-four pigs conducted at the USDA Virology Swine Research Unit, no abnormal clinical signs or cutaneous lesions were observed after either primary or secondary injections of NYVAC-based recombinants (Appendix J). In a total of forty Rhesus macaques inoculated with NYVAC-based recombinants carrying SIV, HIV-1, HIV-2, rabies and hepatitis antigens in different combinations, no local or systemic abnormal clinical signs have been observed (Appendix K). In an immunogenicity study of squirrel monkeys, subcutaneous inoculation with NYVAC-RG (vP879) produced no significant adverse reactions (Appendix L).

3. **Immunogenicity:** Inoculation of mice with NYVAC expressing the HIV-1 (IIIB) envelope glycoprotein elicited humoral responses against the envelope glycoprotein and spleen cell cytolytic reactivity with characteristics of cytotoxic T lymphocytes (Appendix C). In four- to six-week-old mice, NYVAC-RG (vp879) had a PD_{50} value of 3.70 $TCID_{50}$ (Appendix B). In a NYVAC-RG (vP879) dose response using rabbits and four routes of inoculation (ID, SC, IM, and scarification) seroconversion was obtained at all but the lowest dose, and was best in the animals injected IM (Appendix M). NYVAC-based recombinants expressing pertinent immunogens from either pseudorabies virus (PRV) or Japanese encephalitis virus (JEV) have been evaluated in the target species, swine. The swine were protected from a live PRV nasal challenge (Appendices J, N and O), with the NYVAC-based recombinant being as efficacious as commercially available vaccine in preventing clinical disease and viral shedding (Appendix N). Pigs inoculated with a NYVAC-based recombinant against JEV were fully protected from viremia after a live JEV challenge (Appendix N). Subcutaneous inoculation of squirrel monkeys with NYVAC-RG (vP879) produced high titers of rabies neutralizing antibodies after one inoculation, and strong anamnestic responses after the second (Appendix L).

4. **Environmental distribution:** In the above study involving piglets, ten controls were housed with the vaccinates, and remained seronegative through day of challenge (Appendix I). In a separate attempt to isolate the vaccine from site of inoculation, two pigs were injected intravenously with NYVAC (vP866). Blood samples were obtained over times ranging from 5 minutes to 144 hours post-inoculation. NYVAC (vP866) virus was isolated only from the buffy coat and whole blood from the 5 min sample from one pig. No virus was isolated from any other sample (Appendix P).

VIROGENETICS

NYVAC-RG (vP879) is a recombinant rabies vaccine containing the rabies glycoprotein G gene. This vaccine is being proliferated for clinical testing in humans, and an IND application is being prepared for submission to the FDA (Appendix Q).

The USDA/APHIS has assigned Biosafety Level-1 to a contained release involving a NYVAC-based Japanese Encephalitis Virus vaccine (vP908) to be administered to 150 swine at Purdue University (Appendix R).

5. **Geographical distribution:** The NYVAC (vP866) vector has demonstrated the potential to be used as a vaccine for a wide variety of human and veterinary diseases worldwide. The highly attenuated phenotype of the NYVAC vector, coupled with its potency as an immunization vehicle provides a favorable benefit-to-risk ratio. It possesses the advantages of classical vaccinia-based vaccine candidates, yet has attenuation characteristics consistent with an enhanced safety profile.

6. **Recommended NIH/CDC Biosafety Level:** The NIH/CDC recommended Biosafety Level for vaccinia is BL-2. Due to the highly attenuated nature of NYVAC, the absence of animal-to-animal transmission, and the demonstration of significantly limited replicative ability in human cells, we are requesting reduction to Biosafety Level 1.

TAB	SUMMARY OF NYVAC APPENDICES
A	"The Complete DNA Sequence of Vaccinia Virus", Goebel, *et al.*, *Virology*, 179:247-266 (1990).
B	"NYVAC: A Highly Attenuated Strain of Vaccinia Virus", Tartaglia *et al.*, *Virology*, 188:217-232 (1992). This paper presents data on the identity, safety, and potency of NYVAC, and compares it to other strains of vaccinia and the canarypox vector ALVAC (CPpp).
C	"Highly attenuated Poxvirus Vectors in AIDS Vaccine Development", Cox *et al.* Submitted for publication. This paper presents data showing the ability of NYVAC-based constructs to express extrinsic immunogens and elicit protective immune responses.
D	"Vaccinia Virus Host Range Genes", Perkus, *et al.*, *Virology*, 179:276-286 (1990). This article discusses the diminished or eliminated ability of NYVAC to replicate in tissue culture cells derived form certain species, such as swine and humans.
E	This study compares replication of two NYVAC-based recombinants on eight mammalian cell lines, including human MRC-5.
F	This study compares the replicative ability of three vaccinia strains (NYVAC, WR L-variant, and VC-2) by analyzing their dissemination to and accumulation in mouse ovaries, lungs, livers, and spleen.
G	Report on the virulence of NYVAC-RG (vP879) in rabbits inoculated by the intracerebral route (PMs&v report #LV075CRT).
H	Letter from Dr. Fred Quimby, Director, Center for Research Animal Resources, Cornell University; and draft report from Dr. Dorothy Holmes, both describing the absence of any detectable local or systemic reaction in twelve horses vaccinated with NYVAC-based recombinants.
I	Report presenting serology data which addresses the issue of transmission of recombinants from vaccinated swine to non-vaccinates.
J	"Vaccination of Pigs Against Pseudorabies with Highly Attenuated Vaccinia (NYVAC) Recombinant Viruses", Brockmeier, *et al.*, submitted for publication. This paper discusses the safety, potency, and efficacy of a NYVAC-based JEV vaccine.
K	Letter from Dr. Genoveffa Franchini, Head of the Vaccine Unit, Laboratory of Tumor Cell Biology, NIH, NCI.

VIROGENETICS

L	Report on the immune response to NYVAC-RG (vP879) in *Saimiri sciureus* monkeys (PMs&v report #MV022CRT).
M	Report on the virulence and antigenicity of NYVAC-RG (vP879) in the rabbit. Effect of route of inoculation and dose (PMs&v report #LV065CRT).
N	"Novel Poxvirus Strains as Research Tools and Vaccine Vectors", Tartaglia *et al., AIDS RESEARCH REVIEWS*, Vol. 3, Koff, Wong-Staal, Kennedy, eds., Marcel Dekker, Inc., New York, NY, 1993.
O	"Protection of Mice and Swine from Pseudorabies Virus Conferred by Vaccinia Virus-Based Recombinants", Riviere *et al., J. Virology,* 66:3424-3434 (1992).
P	Personal communication between Dr. William Mengeling, USDA National Animal Disease Center, and Arvilla Trag. Typed transcript of letter is included. Photocopy of original fax and coversheet are enclosed for veracity.
Q	Investigator's Brochure: NYVAC-RG (vP879). This document will accompany our IND submission as proscribed in 21 CFR Part 312.
R	A letter from Dr. Cyril Gay of USDA/APHIS, granting Virogenetics a Biosafety Level-1 for a contained release involving a NYVAC-based Japanese Encephalitis Virus vaccine (vP908) to be administered to 150 swine at Purdue University. The letter presents a list of the major considerations used in determination of the Biosafety Level-1.

Federal Register

Wednesday
August 18, 1993

Part XVIII

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Advisory Committee Meeting and Proposed Actions Under the Guidelines for Research Involving Recombinant DNA Molecules; Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee on September 9–10, 1993. The meeting will be held at the National Institutes of Health, Shannon Building, Building 1, Wilson Hall, 3rd Floor, 9000 Rockville Pike, Bethesda, Maryland 20892, starting at approximately 9 a.m. on September 9, 1993, to adjournment at approximately 5 p.m. on September 10, 1993. The meeting will be open to the public to discuss Proposed Actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958) and other matters to be considered by the Committee. The Proposed Actions to be discussed will follow this notice of meeting.

Attendance by the public will be limited to space available. Members of the public wishing to speak at this meeting may be given such opportunity at the discretion of the Chair.

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, Phone (301) 496–9638, FAX (301) 496–9839, will provide materials to be discussed at this meeting, roster of committee members, and substantive program information. Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should contact Dr. Wivel in advance of the meeting. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: August 3, 1993.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 93–20063 Filed 8–17–93; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Proposed Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed actions under the NIH guidelines for research involving recombinant DNA molecules (51 FR 16958).

SUMMARY: This notice sets forth proposed actions to be taken under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958). Interested parties are invited to submit comments concerning these proposals. These proposals will be considered by the Recombinant DNA Advisory Committee (RAC) at its meeting on September 9–10, 1993. After consideration of these proposals and comments by the RAC, the Director of the National Institutes of Health will issue decisions in accordance with the NIH Guidelines.

DATES: Comments received by September 1, 1993, will be reproduced and distributed to the RAC for consideration at its September 9–10, 1993, meeting.

ADDRESSES: Written comments and recommendations should be submitted to Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, or sent by FAX to 301–496–9839.

All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from the Office of Recombinant DNA Activities, Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: The NIH will consider the following actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

I. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Economou, Glaspy

On July 12, 1993, Drs. James S. Economou and John Glaspy of the University of California, Los Angeles, California, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Genetically Engineered Autologous Tumor Vaccines Producing Interleukin-2 for the Treatment of Metastatic Melanoma.

II. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Dr. O'Shaughnessy

In a letter dated September 9, 1992, a letter was received indicating the intention of Dr. Joyce A. O'Shaughnessy, National Institutes of Health, Bethesda, Maryland, to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Retroviral Mediated Transfer of the Human Multi-Drug Resistance Gene (MDR–1) into Hematopoietic Stem Cells During Autologous Transplantation After Intensive Chemotherapy for Breast Cancer.

During the December 3–4, 1992, Recombinant DNA Advisory Committee meeting, approval of the protocol was deferred until the investigators returned to the committee with the following:

(1) Data demonstrating that human CD34(+) cells can be transduced *in vitro* with the actual vector that will be used for the human clinical protocol;

(2) A description of the methods that will be used to monitor gene expression in bone marrow and tumor cells; and

(3) A description of the endpoint for determining bone marrow recovery, i.e., comparison of gene amplification and the rate of polymorphonuclear leukocyte recovery following taxol administration.

On July 14, 1993, Dr. Joyce O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland, resubmitted a human gene

therapy protocol for review and approval.

III. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Das Gupta and Cohen

In a letter dated February 26, 1993, Drs. Tapas K. Gas Gupta and Edward P. Cohen of the University of Illinois College of Medicine, Illinois, and Dr. Jon M. Richards of the University of Chicago, Chicago, Illinois, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Immunization of Malignant Melanoma Patients With Interleukin-2-Secreting Melanoma Cells Expressing Defined Allogeneic Histocompatibility Antigens.*

During the June 7–8, 1993, Recombinant DNA Advisory Committee meeting, approval of the protocol was deferred until the investigators returned to the committee with the following:

(1) Data demonstrating the efficiency of transduction in Mel-4 cells;

(2) Data demonstrating viability, IL–2 production; and *in vivo* murine effect of irradiated transduced cells (either 5,000 or 10,000 rads);

(3) Rationale for ethnic eligibility criteria;

(4) Complete responses to the *Points to Consider*; and

(5) RCR testing data demonstrating safety of the vector preparation.

On July 13, 1993, Drs. Tapas K. Das Gupta and Edward P. Cohen, University of Illinois at Chicago, Chicago, Illinois, resubmitted a human gene therapy protocol for formal review and approval. The revised title of this protocol is: *Pilot Study of Toxicity of Immunization of Patients with Unresetable Melanoma with IL–2 Secreting Allogeneic Human Melanoma Cells.*

IV. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Cassileth, Podack, Sridhar, and Savaraj

In a letter dated December 22, 1992, Drs. Peter A. Cassileth and Eckhard Podack, University of Miami, Miami, Florida, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene Product in Limited Stage Small-cell Lung Cancer.*

During the Match 1–2, 1993, Recombinant DNA Advisory Committee meeting, approval of the protocol was deferred until the investigators returned to the committee with the following:

(1) A definition of the clinical endpoints;

(2) Clonogenic assays using irradiated tumor cells; and

(3) A revised Informed Consent document, including a statement that some patients selected for gene therapy will require a second surgical procedure to obtain material for the study.

In a letter dated July 14, 1993, Drs. Peter Cassileth, Eckhard R. Podack, Kasi Sridhar, and Niramol Savaraj of the University of Miami School of Medicine, Miami, Florida, resubmitted a human gene therapy protocol for formal review and approval.

V. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Kun, Sanford, Brenner, Heideman, Oldfield

On July 12, 1993, Drs. Larry E. Kun, R.A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital Memphis, Tennessee, and Dr. Edward H. Oldfield of the National Institutes of Health, Bethesda, Maryland, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: *Gene Therapy for Recurrent Pediatric Brain Tumors.*

VI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Therapy Protocol/Drs. Wong-Staal, Poeschla

In a letter dated July 14, 1993, Drs. Flossie Wong-Staal, Eric Poeschla, and David Looney of the University of California, San Diego, La Jolla, California, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Phase I Clinical Trial to Evaluate the Safety and Effects in HIV–1 Infected Humans of Autologous Lymphocytes Transduced with a Ribozyme that Cleaves HIV–1 RNA.

VII. Amendment to Appendix D–XXVII of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Greenberg, Riddell

On July 9, 1993, Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, indicated their intention to submit a major modification to a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The current title of this protocol is: A Phase I Study of Cellular Adoptive Immunotherapy Using Genetically Modified CD8+ HIV-Specific T Cells for HIV-Seropositive Patients Undergoing Allogeneic Bone Marrow Transplant. The revised title of this protocol is: Phase I Study to Evaluate the Safety of Cellular Adoptive Immunotherapy using Genetically Modified CD8+ HIV-Specific T Cells in HIV Seropositive Individuals.

VIII. Addition to Appendix D of the NIH Guidelines Regarding Semliki Forest Virus/Dr. Temple

In a letter dated February 8, 1993, Dr. Gary F. Temple of Life Technologies, Inc., Gathersburg, Maryland, submitted a request for a reduction in physical containment from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus (SFV) vector expression system.

During the June 7–8, 1993, Recombinant DNA Advisory Committee meeting, approval was deferred until the investigators returned to the Committee with the following:

(1) A product information sheet informing customers of the potential health risk of the expression system, standard methods to be used for virus inactivation, a helper virus assay to detect SFV, and a description of symptoms and procedures to be followed in the event that SFV infection occurs in a laboratory worker (including methods to prevent transfer to insect vectors and environmental spread); and

(2) SFV inactivation data.

In a letter dated February 8, 1993, Dr. Gary F. Temple of Life Technologies, Inc., Gaithersburg, Maryland, resubmitted a request for a reduction in physical containment from Biosafety Level 3 to Biosafety Level 2 for an SFV vector expression system.

IX. Amendments to Section III and Appendix F of the NIH Guidelines Regarding the Cloning of Toxin Molecules

In a letter dated July 28, 1993, Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland, requested amendments to sections III, IV, and V, and appendices C and F regarding the review process for experiments involving the cloning of toxin molecules.

X. Report on Minor Modifications to NIH-Approved Human Gene Transfer Protocols

Dr. LeRoy Walters, Chair of the Recombinant DNA Advisory Committee, will present an update on minor modifications to NIH-approved human gene transfer protocols.

XI. Working Group Report on Categories of Human Gene Transfer Experiments That Are Exempt From RAC Review/ Dr. Parkman

Dr. Robertson Parkman, Chair of the Working Group on Categorization of Protocols, will present a report on proposed categories of human gene transfer experiments that would be exempt from the RAC review process.

XII. Amendment to Appendix D of the NIH Guidelines Regarding Actions Taken Under the Guidelines

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland, requests an amendment to appendix D. Appendix D is proposed to read:

"Appendix D—Actions Taken Under the Guidelines.

"As noted in the subsection of Section IV–C–1–b–(1), the Director, NIH, may take certain actions with regard to the Guidelines after the issues have been considered by the RAC. An updated list of these actions are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892."

XIII. Amendment to the Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC of the Points to Consider/ NIH Guidelines

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland, requests an amendment to the *Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC* (Federal Register, February 18, 1993, page 9104). This amendment will establish consistency in protocol submissions and will focus oral responses by the principal investigators. The title and section I is proposed to read:

Guidelines for the Submission of Human Gene Transfer Protocols for Review by the Recombinant DNA Advisory Committee

I. Investigator Submitted Material:

Written proposals must be submitted in the following order: (1) Scientific abstract—1 page; (2) non-technical abstract—1 page; (3) IBC and IRB approvals; (4) Points to Consider—5 pages; (6) protocol—20 pages excluding appendices; (7) Informed Consent Document—approved by the IRB; (8) appendices including tables, figures, and manuscripts; and (9) CVs—2 pages in Biosketch format. When a proposal has been submitted previously, there should be a short section (≤200 words) immediately following the abstracts that summarizes the major revisions since the last review. Data provided * * *.

* * * written responses (including critical data in response to the primary reviewers' comments) must be submitted by the Principal Investigators to ORDA ≥2 weeks before the RAC meeting.

Oral Responses to the RAC. Principal Investigators must limit their oral responses to the RAC only to those questions that are raised during the meeting. Oral presentations of previously submitted material and/or critical data that was not submitted ≥2 weeks prior to the RAC meeting is prohibited.

Currently, the title and section I reads:

Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the Recombinant DNA Advisory Committee

I. Investigator Submitted Material:

"Written proposals should begin with the lay and scientific abstracts, followed by the *Points to Consider* and material provided in the body of an R01 format (section A–D). When a proposal has been submitted previously, there should be a short section (≤ 200 words) immediately following the abstracts that summarizes the major revisions since the last review. Length limitations are 4–5 pages for the *Points to Consider*, 2 pages each for CVs (Biosketch format) and 20 pages for the body of the proposal (excluding tables, figures, appendices, and manuscripts). Data provided * * *.

* * * written responses from the Principal Investigators ≥ 2 weeks before the RAC meeting.

Oral Presentations at RAC Meetings provide only a brief overview of the proposal; they should concentrate on questions raised by the reviewers before and at the meeting. Oral presentations should be ≤ 20 minutes: ≤ 10 minutes for the overview and ≤ 10 minutes for responses to the reviewers' questions.

XIV. Amendments to Appendix B of the NIH Guidelines Regarding Updating the Classification of Microorganisms/ Fleming

In a letter dated June 24, 1993, Dr. Diane O. Fleming, President of the Mid-Atlantic Biological Safety Association requests updating Appendix B: *Classification of Microorganisms on the Basis of Hazard.* The Mid-Atlantic Biological Safety Association submitted an updated list of the classification of microorganisms for the committee to review which includes the latest taxomomy and agent risk group classifications as defined by the Centers for Disease Control.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally, NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the *NIH Guidelines.* In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

Daryl A. Chamblee,

Acting Deputy Director for Science Policy and Technology Transfer.

[FR Doc. 93–20064 Filed 8–17–93; 8:45 am]

BILLING CODE 4140–01–M

RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

September 9-10, 1993

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]
September 9-10, 1993

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-fifth meeting at 9:00 a.m. on September 9, 1993, at the National Institutes of Health (NIH), Building 1, Wilson Hall, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee Members:

Nancy L. Buc, Weil, Gotshal, and Manges
Alexander M. Capron, University of Southern California
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
Roy H. Doi, University of California, Davis
Krishna R. Dronamraju, Foundation for Genetic Research
E. Peter Geiduschek, University of California, San Diego
Robert Haselkorn, University of Chicago
Susan S. Hirano, University of Wisconsin
Donald J. Krogstad, Tulane University School of Medicine
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Arno G. Motulsky, University of Washington Medical School
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, VA Polytechnic Institute & State University

Executive Secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment I).

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[1]The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

Non-Voting Agency Representative:

Kurt Gunter, Food and Drug Administration

Liaison Representative:

Daniel Jones, National Endowment for the Humanities

National Institutes of Health Staff:

Bobbi Bennett, OD
Michael Blaese, NCI
Brian Brewer, NHLBI
Sandra Bridges, NIAID
Diane Bronzert, NCI
Chin-Shyan Chu, NHLBI
Sheila Compton, NHLBI
Kenneth Cowan, NCI
Cynthia Dunbar, NHLBI
Maryellen Franko, NCI
Jay Greenblatt, NCI
Barry Goldspiel, NCI
Christine Ireland, OD
Hiroyuki Ishii, NCI
Calvin Jackson, OD
Sachiko Kajigaya, NHLBI
Masako Kawase, NHLBI
Becky Lawson, OD
Rachel Levinson, OD
Julanna Lisziewicz, NCI
Kevin McDonald, NHLBI
Catherine McKeon, NIDDK
Richard Morgan, NCHGR
Koichi Moyamura, NHLBI
Carla Pettineli, NIAID
Joanne Riley, NCI
Nava Sarver, NIAID
Erasmus Schneider, NCI
Lisa Seachrist, NCI
Joyce O'Shaughnessy, NCI
Tom Shih, OD
Brian Sorrentino, NHLBI
Daisy Sun, NCI
Frances Taylor, NINDS
Rosemary Torres, OD
Debra Wilson, OD

Haiping Wu, NHLBI

Others:

W. French Anderson, University of Southern California
Bridget Binko, Cell Genesys, Inc.
G'dali Braverman, Act Up
Shannon Brownlee, U.S. News and World Report
Rachel Carle, Genzyme Corporation
Mike Casey, Genetic Therapy, Inc.
Peter Cassileth, University of Miami School of Medicine
Jan Chappell, Genetic Therapy, Inc.
Fred Chang, University of Michigan
Henry Chang, Shared Medical Research Foundation
Yawen Chiang, Genetic Therapy, Inc.
Edward Cohen, University of Illinois at Chicago
Wanda deVlaminck, Avigen, Inc.
Anne Driscoll, Cowen & Company
Michelle Durand, The French Embassy
James Economou, University of California, Los Angeles Medical Center
Susan Falen, Genetic Therapy, Inc.
Mitchell Finer, Cell Genesys, Inc.
Diane Fleming, MID-Atlantic Biosafety Association
Danielle Foullon, The Pink Sheet
Joyce Frey, Food and Drug Administration
Morgan Gale, National Narrowcast
Richard Giles, MD Anderson Cancer Center
Douglass Given, Progenitor, Inc.
Phillip Greenberg, University of Washington
David Holzman, BioWorld
Thomas Horiagon, Chimerix, Inc.
John Jaugstetter, Genentech, Inc.
Susan Jenks, Journal of the National Cancer Institute
Michael King, Alex Brown & Sons
Toshihiko Komori, Chugai Pharma U.S.A., Inc.
Steven Kradjian, Vical, Inc.
John Krauss, University of Michigan Medical Center
Larry Kun, St. Jude Children's Research Hospital
Donald Longenecker, Viagene, Inc.
Daniel Maneval, Canji, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Bruce Merchant, Viagene, Inc.
Noel Messenger, Applied Immune Sciences, Inc.
Robert Moen, Genetic Therapy, Inc.
Elaine Morikawa, TV Asahi
Arthur Nienhuis, St. Jude Children's Research Hospital

Jeffrey Ostrove, Microbiological Associates, Inc.
Robert Overell, Targeted Genetics Corporation
Seth Pauker, Parexel International
Liz Pennisi, Science News
Anne Petruska, FDC Reports
Stephen Pijar, University of Maryland, Baltimore
Doros Platika, Progenitor, Inc.
Eckhard Podack, University of Miami School of Medicine
Eric Poeschia, University of California, San Diego
Lisa Raines, Genzyme Corporation
Urbin Ramstebt, Virus Research Institute
Paul Recer, Associated Press
Thomas Reynolds, Targeted Genetics Corporation
Rex Rhein, Biotechnology Newswatch
John Richards, University of Illinois at Chicago
Phil Richards, Public
Stanley Riddell, Fred Hutchinson Cancer Research Center
Bruce Schackman, Furman Selz, Inc.
Bill Schwieterman, Food and Drug Administration
Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambience Awareness International, Inc.
Marcus Stern, Copley Newspapers
Frank Sturtz, Progenitor, Inc.
Nevin Summers, Massachusetts Institute of Technology
Colleen Sundstrom, Howard University
Gary Temple, Life Technologies, Inc.
Larry Thompson, Medical News Network
George Thornton, The R.W. Johnson Pharmaceutical Research Institute
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Joseph Van Houten, The R.W. Johnson Pharmaceutical Research Institute
Christine Vanderpol, Rhone-Poulenc Rorer
Ashley Wivel, Public
Flossie Wong-Staal, University of California, San Diego
Mang Yu, University of California, San Diego

I-A. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notice of the meeting was published in the *Federal Register* on August 18, 1993 (58 FR 44098), as required by the *National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the protocol, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, followed by the public at large.

I-B. UPDATE ON THE LETTER SENT TO THE NIH DIRECTOR REGARDING COMPENSATION FOR RESEARCH-RELATED INJURIES/DR. WALTERS

Dr. Walters summarized the events surrounding the RAC's recommendations to the NIH Director regarding the provision of medical care to subjects who may be injured in the course of their participation in clinical research. Dr. Walters explained that at the time of the June 7-8 RAC meeting, he had not received a response to his letter sent to Bernadine Healy, former NIH Director on January 6, 1993; therefore, the committee recommended that a follow-up letter should be sent to the NIH Director regarding this issue.

Dr. Walters described the correspondence that had been received since the June 7-8 meeting: (1) a letter from Dr. Bernadine Healy dated June 3, and (2) a letter from the Acting NIH Director, Dr. Ruth Kirschstein, dated August 6. Dr. Healy's letter stated the following:

> "I understand the RAC's present interest in seeing greater policy consistency among research institutions in this area, but I do not believe we can achieve a more uniform approach without also addressing the central issues involved in an expanded Federal role. Consequently, before another panel is formed to study this issue anew, I believe it is necessary to establish that the need in this area is greater today than 10 years ago. Have the frequency and incidence of non-negligent injuries increased? Is the number of research subjects who are not being treated or compensated on the rise? Are research subjects more concerned about and reluctant to participate in research today?
>
> "Further review of the compensation question will be included in the responsibilities of the NIH Science Policy Studies Center. Recently established to enhance NIH's capacity to identify and analyze the social, legal, ethical, and economic implications of biomedical and behavioral research, the Center is planning a priority setting conference in the fall to establish criteria for addressing pending and emerging issues. This broad assessment of current issues may give the NIH a better understanding of the compensation issue's relative pervasiveness and importance. By the end of the year, we may also have a clearer picture of

the national health care reform effort and whether it might result in health insurance or other changes that will be relevant to this issue. As we continue to gather more information, the NIH would welcome any additional information or insights the RAC may have in this matter."

Dr. Kirschstein's letter addressed the concerns of the RAC in the following statement:

"Given NIH's peripheral role in the reform effort, I am transmitting the RAC's recommendation to the Assistant Secretary for Health with a request that the specific issue of covering research injuries be brought to the attention of the National Task Force on Health Care Reform."

I-C. EXECUTIVE SECRETARY REPORT ON POSSIBLE ADVERSE EFFECTS/ DR. WIVEL

Dr. Wivel noted that a written report of possible adverse effect was submitted by Dr. Edward Oldfield, NIH, Bethesda, Maryland, in a patient enrolled in his human gene transfer protocol entitled: "Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir." Dr. Wivel summarized a verbal report by Dr. Crystal to the NIH Institutional Biosafety Committee (IBC) regarding a possible adverse effect in a patient enrolled in his human gene transfer protocol entitled: *A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.*

I-D. RECOGNITION FOR SERVICE ON THE RAC/DR. WALTERS

Dr. Walters recognized several members of the RAC whose term has expired. He expressed the gratitude of the committee for their years of service, dedication, and careful review of recombinant DNA research. The outgoing members of the RAC are: Drs. Carmen, Geiduschek, Hirano, Krogstad, and Post. Outgoing RAC members will continue to serve on the committee until future appointments have been finalized by the Department of Health and Human Services.

II. MINUTES OF THE JUNE 7-8, 1993, RAC MEETING

Dr. Walters called on Dr. DeLeon to review the minutes of the June 7-8, 1993, RAC meeting. Dr. DeLeon stated that the minutes were an accurate reflection of the June meeting. Minor corrections were submitted by Drs. DeLeon, Smith, and Parkman.

Committee Motion

The RAC approved a motion made by Dr. DeLeon and seconded by Dr. Parkman to accept the June 7-8, RAC minutes with the inclusion of minor grammatical changes by a vote of 18 in favor, 0 opposed, and no abstentions.

III-A. CHAIR REPORT ON RAC-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters summarized the human gene transfer protocols that have been approved by the RAC to date. Of the 52 protocols approved by the RAC, 46 of these studies (24 gene therapy and 22 gene marking) have been approved by the NIH Director. Listed below is a breakdown of the NIH- and RAC-approved protocols (Attachment II):

* 18/Cancer
* 2/Human immunodeficiency virus (HIV)
* 9/Genetic diseases (5/cystic fibrosis, 1/adenosine deaminase deficiency (ADA), 1/familiar hypercholesterolemia, and 2/Gaucher disease)
* 21/Others

III-B. CHAIR REPORT ON MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters stated that a total of 18 minor modifications have been approved to date. He summarized three modifications that were approved since the June 7-8, RAC meeting: (1) Dr. James Wilson has been granted permission to transfer his 2 protocols from the University of Michigan to the University of Pennsylvania, and (2) Dr. Scott Freeman has been granted permission to transfer his protocol from the University of Rochester to Tulane University School of Medicine (Attachment III).

IV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *PHASE I STUDY OF TRANSFECTED CANCER CELLS EXPRESSING THE INTERLEUKIN-2 GENE PRODUCT IN LIMITED STAGE SMALL-CELL LUNG CANCER*/DRS. CASSILETH, PODACK, SRIDHAR, AND SAVARAJ

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol resubmitted by Drs. Peter A. Cassileth, Eckhard Podack, and Kasi Sridhar of the University of Miami, and Dr. Niramol Savaraj of the Miami Veterans Administration Hospital, Miami, Florida. This protocol was deferred at the March 1993, RAC meeting until the investigators returned to the full RAC with additional data and a revised protocol. The intent of this protocol is to culture small-cell lung carcinoma (SCLC) cells from patients and infect them with a bovine papilloma virus (BPV) vector expressing the gene for interleukin-2 (IL-2). Following lethal irradiation, IL-2 producing tumor cells will be reimplanted into patients to stimulate proliferation of tumor-specific cytotoxic T-lymphocytes (CTL) capable of destroying the reimplanted and established tumors. One primary concern of Dr. Miller was whether irradiation will prevent growth of the modified tumor cells after injection into patients. This issue is critical because the addition of BPV transforming genes into tumor cells could possibly increase the

tumorigenicity of these cells. Data demonstrate that no detectable cells (< 1 in 7×10^6 cells) survive following lethal irradiation. In his primary review, Dr. Miller questioned whether it is adequate to irradiate tumor cells at 6,000 rad for the human trial while in preclinical studies, data were obtained using 12,000 rad irradiation. The investigators responded to Dr. Miller's concerns stating that 12,000 rad will be used for the human study. Dr. Miller expressed satisfaction with the PI's response.

Review--Dr. Haselkorn

Dr. Haselkorn stated that the investigators have provided data demonstrating that the irradiation schedule for the transfected cells is sufficient to inhibit proliferation of clonogenic cells while retaining their ability to express IL-2. Dr. Haselkorn said that he had solicited the expert opinion of Dr. Lou Laimins of the University of Chicago, regarding the BPV vector. Dr. Laimins questioned the investigators' assumption that the BPV vector is maintained as an episome at high copy number in transfected human tumor cells and that high level IL-2 production is facilitated. Dr. Laimins notes that while BPV plasmids transfected into mouse cells can be maintained as episomes, BPV usually integrates into human chromosomes. Therefore, the BPV vector demonstrates no advantage over retrovirus vectors. Dr. Haselkorn questioned whether the BPV vector actually expresses higher levels of IL-2 than retrovirus vectors.

Review--Dr. Secundy

Dr. Secundy stated that her comments were primarily confined to the Informed Consent document. Most of her initial concerns about the discrepancy between the gene therapy description in the protocol versus the Informed Consent document have been corrected. One remaining concern is the statements regarding third party payer reimbursement for research related injuries in Section 4.7 of the protocol and the Informed Consent document. The responsibilities for the financial burden must be clarified.

Other Comments

Dr. Walters summarized written comments submitted by Dr. Smith concerning: (1) a discrepancy between the dose of radiation used for the transfected cells versus the dose proposed for the clinical study, and (2) a discrepancy in the data regarding the success rate of growing SCLC cells *in vitro*. One statement refers to a 70% success rate versus a 30% success rate stated elsewhere in the protocol.

Dr. Geiduschek expressed his concern about the episomal status of the BPV vector within transfected cells and the levels of IL-2 production. Dr. Miller stated that the issue of whether the vector is episomal or integrated is irrelevant since the investigators have demonstrated high level IL-2 production in transfected cells. Dr. Post suggested that the RAC should stipulate a minimum level of IL-2 production for cells to be administered to the patients.

Investigator Response--Drs. Podack and Cassileth

Responding to the question of IL-2 production, Dr. Podack stated that in one human cell line studied, the level of production was between the range of 6,000 and 9,600 units of IL-2/ml/10^6 cells/24 hours; and in another cell line, the level of expression was between 4,000 and 5,000 units of IL-2/ml/10^6 cells/24 hours. These levels are at least one order of magnitude higher than the same cell lines transfected with retrovirus vectors. After irradiation, the transfected cells continued to produce the same level of IL-2 for 2 days; however, IL-2 expression declined over the next 6 days. Regarding the episomal status of the BPV vector, he explained that the vector is maintained episomally at approximately 16 copies per cell in human HeLa cells. Data derived from murine experiments suggest that there is a strong correlation between copy number and the levels of IL-2 expression.

Dr. Podack stated that Dr. Savaraj, a co-investigator on this protocol, has established cell lines from fresh tumor specimens at approximately a 70% success rate. Dr. Podack explained that most of the preclinical data was obtained using a cell line that has been grown for a relatively short term *in vitro*. This cell line was established in approximately 2 months, and aliquots of these cells were cryopreserved. All experiments were performed using cells grown from the cryopreserved stock. Dr. Podack stated that more comprehensive data would be obtained from a broader range of cell lines established from fresh tumors.

Dr. Cassileth responded to questions relating to the clinical aspects of the protocol. The financial responsibility for costs associated with the treatment of adverse effects arising from the research study will be the responsibility of the patient, third party payers, or both. He explained that his local Institutional Review Board (IRB) requires this statement in the Informed Consent document. He said he is of the opinion that compensation for research related injury is an issue that should be addressed in a broader sense.

In regard to their success rate for establishing fresh tumor cell lines, Dr. Cassileth stated that they have demonstrated a success rate of between 70 and 80%. Overall, approximately 30% of the patients initially entered onto the protocol will be eligible to receive the transfected cells due to the exclusion of some patients based on entrance criteria.

Mr. Capron remarked that the terms "therapy" and "treatment" in the Informed Consent document are inappropriate for a Phase I study. Dr. Cassileth agreed to revise this inappropriate language.

Drs. Parkman and Post requested that a minimum level of IL-2 production should be demonstrated prior to reimplantation of the transfected cells into patients. Dr. Miller suggested that 10 units of IL-2/ml/10^6 cells/24 hours is an acceptable minimal level. Dr. Podack stated that 50 units of IL-2/ml/10^6 cells/24 hours is an acceptable minimal level of IL-2 expression.

In regard to Dr. Haselkorn's concern about whether BPV is maintained episomally in

human cells, Dr. Podack stated that data derived from human cell lines, in addition to HeLa, indicate episomal presence based on high level IL-2 production. The level of IL-2 expression is a more pertinent parameter for the present study. Dr. Miller agreed with Dr. Podack's statement. Dr. Miller said that the risk of episomal DNA inadvertently infecting other cells, even if the cells carrying the DNA have been lethally irradiated, is quite remote.

Committee Motion

A motion was made by Dr. Miller and seconded by Mr. Capron to approve the protocol contingent upon review and approval of data demonstrating that cells obtained from patients can be successfully transduced with the proposed vector and that these transduced cells secrete ≥ 50 units of IL-2/ml/10^6 cells/24 hours. The motion passed by a vote of 18 in favor, 0 opposed, and no abstentions.

V. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *RETROVIRAL MEDIATED TRANSFER OF THE HUMAN MULTI-DRUG RESISTANCE GENE (MDR-1) INTO HEMATOPOIETIC STEM CELLS DURING AUTOLOGOUS TRANSPLANTATION AFTER INTENSIVE CHEMOTHERAPY FOR BREAST CANCER*/DR. O'SHAUGHNESSY

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol resubmitted by Dr. Joyce A. O'Shaughnessy, NIH, Bethesda, Maryland. This protocol was deferred at the December 1992 RAC meeting until the investigators could return to the full RAC with the following: (1) data demonstrating that human CD34(+) cells have been transduced *in vitro* with the vector that will be used in the clinical protocol; (2) a description of the assays that will be used to measure gene expression, and demonstrate how this expression will be monitored in bone marrow and tumor cells; and (3) a description of the endpoint for determining efficacy (evaluation criteria), i.e., comparison of gene amplification and the rate of white blood cell recovery following Taxol treatments one and three.

Dr. Parkman stated that the transplantation procedure is part of a freestanding clinical protocol that deals with autologous transplantation for patients with responsive Stage IV breast cancer following chemotherapy with ifosfamide, carboplatin, and etoposide. Patients will be transplanted with a combination of bone marrow and peripheral blood stem cells and will receive subsequent Taxol administration. The gene transfer portion of the proposed study is the only aspect of the protocol that requires RAC review.

CD34(+) stem cells will be isolated from bone marrow and peripheral blood. If adequate numbers of these cells are obtained, a portion will be transduced *in vitro* with a retrovirus vector expressing the MDR-1 gene. Both transduced and untransduced CD34(+) cells will be reinfused back into the patients. Patients demonstrating

subsequent progressive disease will receive Taxol administration on a dose-escalation schedule. Although the protocol has adequate preclinical data, a major problem raised in the last review was that most preclinical data were obtained employing a vector based on the Harvey murine sarcoma virus, which was different from the vector proposed for the clinical trial. In this resubmission, the PI has addressed all the significant questions raised in the last review including: (1) construction and evaluation of the Moloney murine leukemia virus based vector, G1MD, (2) demonstration of the ability of this vector to transduce the target human CD34(+) cells, (3) demonstration of the transduced MDR-1 gene in primary hematopoietic cells, (4) demonstration of expertise in the polymerase chain reaction (PCR) assay of MDR-1 gene expression, and (5) clinical evidence for the ability to transduce CD34(+) cells with a vector containing a neomycin resistance (neo^R) gene. The investigators have deleted one of their previous objectives, namely, demonstrating the therapeutic benefit of MDR-1 transduction and have focused instead on demonstration of persistence and proliferation of MDR-1 transduced cells following Taxol administration. Dr. Parkman recommended approval of the revised protocol.

Review--Dr. Brinckerhoff (presented by Dr. Parkman)

In her written comments, Dr. Brinckerhoff explained that this revised protocol is improved over the original submission and directly addresses most of the RAC's previous concerns. She noted two reservations: (1) Since breast cancer patients demonstrate a high incidence of bone marrow metastasis, what percentage of patients will be ineligible to participate in this study? Dr. Parkman explained that breast cancer cells do not express the CD34 marker; therefore, isolation of CD34(+) bone marrow cells employing a CD34 monoclonal antibody column will eliminate most contaminating metastatic breast cancer cells. Dr. Parkman noted that this issue has been raised during the review of previously approved protocols. (2) There is a lack of preclinical data demonstrating long-term expression of the transduced MDR-1 gene in an animal model.

Review--Mr. Capron

Mr. Capron stated that the investigators have adequately responded to the suggested changes in the Informed Consent document. Except for a few typographical errors, the revised Informed Consent is acceptable. Mr. Capron recommended approval of the protocol.

Other Comments

Dr. Miller mentioned that some preclinical data were not included in his review materials and asked the investigators to present their data. Dr. Post asked the investigators to explain in detail the problem of MDR-1 mRNA splicing and the two different gene products coded for by these spliced MRNA species. Dr. Post noted that the stem cell factor used for most of the preclinical studies will not be used in the clinical protocol. Will omission of this factor affect the experimental outcome?

In regard to the issue of compensation for research-related injury, Dr. Walters noted that a statement is included in the Informed Consent document explaining that the NIH Clinical Center will provide short-term medical care, but not long-term care, for physical injuries resulting from a patient's participation in research. This statement is a modification over the usual statement that no care or compensation will be provided.

Investigator Response--Drs. Neinhuis and Sorrentino

In response to Dr. Post's comments about the use of stem cell growth factor, Dr. Neinhuis stated that stem cell growth factor will be used in the human clinical trial due to the fact that it has recently become available. Responding to the question of MDR-1 MRNA splicing, Dr. Neinhuis said that the protein encoded by the spliced MRNA is a small truncated variant gene product and is presumably nonfunctional. Approximately 50% of the MDR-1 gene transcript from the DNA construct based on either the Harvey or Moloney vector is spliced. Both full length and spliced RNA species are co-packaged as virions and are transferred to target cells. mRNA expression of the normal genomic MDR-1 gene, however, is not similarly spliced.

Dr. Sorrentino summarized preclinical studies with the murine model. While earlier studies were performed using a Harvey-based vector, recent results were obtained with the new Moloney-based construct, G1MD. A high titer amphotropic producer clone of the G1MD vector has been isolated from the PA317 packaging cell line. Transfer of the MDR-1 gene to the target cells was assayed by the rhodamine efflux assay. Expression of the transduced MDR-1 gene yielded a "dull" phenotype as compared to a "bright" phenotype for untransduced cells as observed under a fluorescence microscope. These two types of cells were fractionated by a fluorescence activated cell sorter (FACS). MDR-1 gene expression by the G1MD construct in mouse hematopoietic cells was demonstrated. In the *in vivo* murine model, Taxol conferred drug resistance to hematopoietic cells. Data also demonstrated MDR-1 gene expression after transduction with the clinical grade G1MD vector in human CD34(+) cells. MDR-1 proviral DNA was also demonstrated. Quantitative assays for the transduced MDR-1 gene were performed in primates.

Dr. Sorrentino said that similar mRNA splicing of the MDR-1 gene probably occurred in constructs used in other previously approved protocols. Mutations made to eliminate the splice donor and acceptor sites resulted in a construct that expressed the encoded MDR-1 gene, but the vector RNA was unable to be packaged in the producer cell. Presumably all packaging cells produced viral particles containing both RNA species.

Responding to Dr. Miller's question about the replication competent retrovirus (RCR) testing of the clinical grade vector, Dr. Neinhuis said that Genetic Therapy, Inc., has performed extensive testing to confirm the absence of RCR.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Miller to approve the

protocol. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

VI. MAJOR AMENDMENT TO APPENDIX D-XXVII OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *PHASE I STUDY TO EVALUATE THE SAFETY OF CELLULAR ADOPTIVE IMMUNOTHERAPY USING GENETICALLY MODIFIED CD8(+) HIV-SPECIFIC T CELLS IN HIV SEROPOSITIVE INDIVIDUALS*/DRS. GREENBERG AND RIDDELL

Review--Dr. Straus

Dr. Walters noted that Dr. Miller will abstain from discussion and voting on this protocol due to a conflict of interest (employed by the same institution). Dr. Walters called on Dr. Straus to present his primary review of the request for a major amendment to the human gene transfer protocol submitted by Drs. Phillip Greenberg of the University of Washington and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington. This protocol represents a modification of a previously approved Phase I study to evaluate the safety of adoptive immunotherapy of autologous genetically-modified CD8(+) HIV-specific T cell clones in HIV infected individuals with lymphoma following bone marrow transplantation (BMT). Apparently, the accrual from that study has been slower than expected; but the preliminary data suggest that competent cells can be detected in the recipients. The present modification will allow the investigators to accrue HIV-seropositive individuals who lack opportunistic infections, have CD4 cell counts between 200 and 500, do not have lymphoma, and who are not undergoing BMT.

As in the previously approved protocol, the patient's T cells will be modified by a retrovirus containing a hybrid insert of the hygromycin resistance (Hy^R) gene for marking as well as the herpes simplex virus thymidine kinase (HSV-tk) gene. The HSV-tk suicide gene will provide a safety feature that allows the transduced cells to be eliminated with the antiviral drug, ganciclovir, if adverse effects are encountered. This latter concept will be tested in a group of 3 patients enrolled in this study. Dr. Straus stated that this protocol is well developed with the vector and the strategy thoroughly described. He recommended approval of this modification.

Review--Dr. Leventhal (presented by Dr. Straus)

Dr. Leventhal's written comments raised several questions that have been adequately addressed by the investigators. The investigators accepted her suggestion that ganciclovir should be administered in the hospital for the first two days in the event that the treatment exhibits an unanticipated toxic effect. She asked about the necessity for lymph node biopsies prior to treatment. The procedures described for assessing neurologic and pulmonary toxicity are inadequate and more extensive evaluations should be performed. Any adverse effects, if encountered, should be reported to the RAC as well as the IRB. She questioned the future plans for evaluation of this technique. How will the investigators proceed if they find that the treatment is neither toxic nor effective? She noted that her comments were minor, and she recommended approval of this major

amendment.

Review--Dr. Carmen

Dr. Carmen commented that the Informed Consent document is lucid and well written. He suggested a minor revision that would more succinctly inform the patients about this study. The investigators agreed to incorporate Dr. Carmen's suggestion.

Other Comments

Dr. Walters summarized Ms. Meyers' written comments about the Informed Consent document: (1) Will a request for autopsy be included? and (2) Will the patient's medical records be made available to other physicians, NIH, and others? Mr. Capron asked the investigators why they did not apply for a certificate of confidentiality under Section 301(d) of the Public Health Service Act as suggested by their IRB. Dr. Post asked the investigators to summarize the status of patients who were enrolled in the previous study.

Mr. G'dali Braverman from ACT UP expressed his disappointment that patients with CD4 counts below 200 will be excluded from participation in this study, but he was pleased that patients receiving antiviral treatment will not be excluded.

Investigator Response--Dr. Greenberg

Dr. Greenberg explained that the existing protocol has very stringent entrance criteria, i.e., only patients with HIV-related lymphoma receiving BMT are eligible. A total of 8 patients have been evaluated to date, and only 4 have had human leukocyte antigen (HLA) matches for BMT. Of the 4 eligible patients, only one was treated. The other 3 patients had rapidly progressive lymphoma relapse which prevented their entrance into this study. This problem of low patient accrual is the reason for the present amendment. Preliminary data on the one treated patient indicates that CTL activity to HIV can be reconstituted. Similar activity directed toward cytomegalovirus (CMV) infection has been demonstrated previously in a much larger study. Unfortunately, the single HIV patient who received this treatment, died of severe graft-versus-host (GVH) disease as a consequence of the transplantation procedure. No long-term efficacy data are available.

Dr. Greenberg explained that the rationale behind selecting HIV seropositive individuals, who have CD4 counts between 200 to 500, is that this group of patients elicit a better CD8 response and demonstrate fewer complications associated with disease progression. Therefore, a more definitive evaluation of safety and toxicity is possible. In regard to the issue of patient confidentiality, he cannot guarantee that every patient will be codified; however, he is applying for a certificate of confidentiality as noted by Mr. Capron.

Dr. Chase inquired about the rationale for selecting the sample size of 15. Dr. Riddell said that this number was chosen based on other similar Phase I toxicity studies. The primary objective of this study is to determine a dose range that is safe and biologically

relevant. Dr. Parkman inquired about the number of cells that were used for the previous CMV study. Dr. Riddell answered that the highest dose of cells administered was 1 x 10^9 cells/m^2, and this dose was well tolerated. With regard to the future course of this study, Dr. Riddell explained that if no toxicity or positive antiviral effects are observed, the study will progress to a Phase II efficacy trial that will involve a statistically meaningful cohort of patients.

Mr. Capron suggested the addition of a sentence to the Informed Consent document that explains the use of the suicide gene more clearly. The investigators appreciated Mr. Capron's comments and agreed to incorporate the suggested change.

Committee Motion

A motion was made by Dr. Straus and seconded by Mr. Capron to approve this major amendment. The motion passed by a vote of 16 in favor, 0 opposed and 2 abstentions (Drs. Miller and Motulsky abstained from voting because they are employed by the University of Washington).

VII-A. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENE THERAPY FOR RECURRENT PEDIATRIC BRAIN TUMORS*/DRS. KUN, SANFORD, BRENNER, HEIDEMAN AND OLDFIELD

Review--Dr. Geiduschek

Dr. Walters called on Dr. Geiduschek to present his primary review of the protocol submitted by Drs. Larry E. Kun, R. A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the NIH, Bethesda, Maryland. This gene therapy protocol involves HSV-tk gene transduction of brain tumor cells followed by administration of the antiviral drug ganciclovir. This Phase I trial will involve 6 children > 3 years of age with progressive or recurrent malignant supratentorial brain tumors that are resistant to standard therapy. Patients will receive multiple intratumoral injections of between 10^8 and 10^9 murine cells (PA317) in a volume of 1 to 20 milliliters. Patients will be injected with the PA317 vector producing cells (VPC) containing the G1TKSvNa retrovirus vector. Seven days post injection, patients will receive ganciclovir administration for the ablation of transduced tumor cells. Anti-tumor responses will be monitored for more than one year by magnetic resonance imaging (MRI). This Phase I toxicity study is similar to other protocols that have previously been approved by the RAC except for a variation in the surgical procedures that are proposed to deliver the VPC. Dr. Geiduschek noted his concern about the repetitiveness of such studies. Is the public and the progress of gene therapy at this stage best served when a substantial fraction of the still small group of investigators focus quasi-repetitively on a single idea?

Review--Dr. DeLeon

Dr. DeLeon stated that since the proposed vector, protocol, and target cells have been previously reviewed and approved by the RAC for other protocols, there are no major new issues for the RAC to discuss. Dr. DeLeon emphasized that the RAC should consider quality control standards and adopt guidelines for conducting similar types of trials at multiple sites.

Review--Ms. Meyers (presented by Dr. Walters)

Ms. Meyers' written comments raised several questions about the Informed Consent documents. Although they are generally well written, they should include information about the additional medical costs associated with participation in this study, long-term follow-up, other available experimental therapies, and a request for autopsy.

Other Comments

In his written comments, Dr. Smith raised the question of possible adverse effects in relation to the "asymptomatic gliosis" detected by MRI on one of the patients enrolled in Dr. Oldfield's protocol. This observation should be discussed in connection with the present protocol.

Mr. Capron commented that the use of the term "you/your child" in the parent copy of the Informed Consent document is confusing. The way the document reads, it is unclear whether the parent or the child is undergoing treatment. Such documents would be more appropriately written separately in language that is understandable to each person signing the document, i.e., the parents, children 7-14 years of age, and children ≥ 15 years of age.

Dr. Chase remarked that the investigators' response to comments by Ms. Meyers and Dr. Smith were inadequate. Dr. Parkman asked the investigators to elaborate on the inclusion criteria, i.e., the size of tumors and treatment of single versus multiple lesions.

Investigator Response--Dr. Kun

Responding to Dr. Geiduschek's question regarding the similarity of this proposal to other RAC-approved protocols, Dr. Kun explained that a given therapy may have different responses and toxicities depending upon the age of the patient. The present protocol targets childhood instead of adult tumors. This proposal differs from the previously approved pediatric brain tumor protocol submitted by Dr. Culver, et. al., in that the VPC will be stereotactically injected into the tumor mass rather than into an intracystic tumor cavity. Anatomical differences of the injection sites may produce different responses.

In response to several Informed Consent document issues, Dr. Kun said that patients ≥ 18 years of age can legally give consent to participate in a protocol. "You/your child" would mean "you/yourself" for patients over 18, and for patients below this age, it would mean "your child". The simpler assent form is to be signed by children under 18 with

their parents. Mr. Capron and Ms. Buc questioned this practice. Dr. Secundy stated that she is not satisfied with the consent process for minors. Dr. Miller suggested that the forms should be separated for patients under or over 18 years of age. Dr. Kun agreed in principle to change the Informed Consent document stipulated by the RAC; however, such a change would be subject to local IRB approval. This issue of complexity and conformity of Informed Consents evolved into a lengthy discussion to be summarized below (see VII-B: RAC Recommendation Regarding the Establishment of a Working Group to Develop Guidelines for Informed Consent Document).

Responding to Dr. Chase's comment, Dr. Kun noted that all the pertinent changes have been made in the protocol and consent documents. Regarding Dr. Parkman's question about inclusion criteria, Dr. Kun clarified that patients with nonresectable single foci > 1.5 cm and < 5 cm will be included in the study. As to the question of quality control in this multicenter trial, Dr. Kun said that the same standards will apply as for Dr. Oldfield's protocol at NIH. Dr. Kun agreed to include a request for autopsy into the revised Informed Consent document.

Dr. Kun responded to Dr. Smith's inquiry about the changes observed on an MRI obtained on a patient enrolled on Dr. Oldfield's study. Dr. Miller stated that these preliminary results have been overinterpreted in the media, raising false expectations for prospective patients. Dr. Krogstad said the RAC may have to deal with the issue of how preliminary results of gene therapy trials are reported by the media. Dr. Chase shared the same sentiment and urged that the Informed Consent document should properly convey the experimental nature of this kind of therapy to the patients. Dr. Kun said that the present protocol is a very limited Phase I toxicity trial; it is not designed to assess efficacy, which would require a statistically meaningful cohort of samples. The scientific results will be evaluated in concert with the NIH investigators and will avoid conflict of interest with the sponsoring company. Dr. McGarrity of Genetic Therapy, Inc., agreed that independent evaluation of the scientific results is essential.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Motulsky to approve the protocol. The motion passed by a vote of 17 in favor, 0 opposed and no abstentions. A friendly amendment was proposed by Dr. Miller and accepted by Drs. Post and Motulsky. This amendment stipulates that the RAC strongly recommends that the Informed Consent document should be divided into separate documents: (1) a guardian assent form for patients < 18 years of age, (2) a patient assent form for patients < 18 years of age, and (3) a patient consent form for patients ≥ 18 years of age.

VII-B. RAC RECOMMENDATION REGARDING THE ESTABLISHMENT OF A WORKING GROUP TO DEVELOP GUIDELINES FOR INFORMED CONSENT DOCUMENTS

Discussion

During the review of Dr. Kun's brain tumor protocol, several questions regarding

Informed Consent documents and the informed consent process were raised.

Dr. W. French Anderson, University of Southern California, Los Angeles, California, stated that the RAC possesses unique expertise and is qualified to propose policy on scientific and biotechnology issues, as well as informed consent issues relating to human gene transfer. Dr. Anderson suggested that the RAC formulate informed consent recommendations to be communicated to the local IRBs through NIH's Office for Protection from Research Risks (OPRR). Dr. Parkman agreed that such a process would be a useful mechanism. Ms. Buc commented that it would be impractical to develop a universal Informed Consent document that would be applicable to all protocols. A set of common guidelines would be more feasible. Dr. Motulsky suggested that some social science studies should be conducted to obtain practical information on the effectiveness of the informed consent process for complicated genetic diseases.

Dr. Zallen commented on the importance of the Informed Consent document. She agreed that the RAC is in a position to provide useful suggestions based on its extensive expertise in the review of such documents.

Committee Motion

A motion was made by Dr. Krogstad and seconded by Dr. Zallen that the RAC establish a working group to frame pertinent questions relevant to Informed Consent documents and that the Director of OPRR, NIH, should be invited to the December 1993 RAC meeting to address these issues. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

VIII. AMENDMENT TO THE *GUIDELINES FOR THE SUBMISSION OF HUMAN GENE TRANSFER/THERAPY PROTOCOLS FOR REVIEW BY THE RAC* OF THE *POINTS TO CONSIDER/NIH GUIDELINES*/DR. WIVEL

Dr. Wivel presented amendments to the *Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC* (*Federal Register*, February 18, 1993, page 9104). These amendments will establish consistency in the submission of human gene transfer protocols for RAC review and require PIs to focus their oral responses to the RAC's questions and comments. The Title and Section I will read:

> *"Guidelines for the Submission of Human Gene Transfer Protocols for Review by the Recombinant DNA Advisory Committee.*
>
> "I. Investigator Submitted Material:
>
> "Written proposals must be submitted in the following order: (1) scientific abstract--1 page; (2) non-technical abstract--1 page; (3) IBC and IRB approvals; (4) Points to Consider--5 pages; (6) protocol--20 pages excluding appendices; (7) Informed Consent document--approved by the IRB; (8) appendices including tables, figures, and manuscripts; and (9) CVs--2 pages

in Biosketch format. When a proposal has been submitted previously, there should be a short section (≤ 200 words) immediately following the abstracts that summarizes the major revisions since the last review. Data provided....

"...written responses (including critical data in response to the primary reviewers' comments) must be submitted by the Principal Investigators to ORDA ≥ 2 weeks before the RAC meeting.

"Oral Responses to the RAC. Principal Investigators must limit their oral responses to the RAC only to those questions that are raised during the meeting. Oral presentations of previously submitted material and/or critical data that was not submitted ≥ 2 weeks prior to the RAC meeting is prohibited."

Committee Motion

A motion was made by Dr. Krogstad and seconded by Dr. Parkman to accept the amendments to the *Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC.* The motion passed by a vote of 16 in favor, 1 opposed, and no abstentions.

IX. WORKING GROUP REPORT ON CATEGORIES OF HUMAN GENE TRANSFER EXPERIMENTS THAT ARE EXEMPT FROM RAC REVIEW/DR. PARKMAN

Presentation--Dr. Parkman

Dr. Parkman said that the RAC is currently realizing a proliferation in the number of gene transfer protocols submitted for review due to the evolution and maturation of the field of gene therapy. The RAC formed a working group to formulate categories of gene transfer protocols that may not require full RAC review or are considered exempt from RAC purview. Dr. Parkman reported on a tentative consensus that resulted from a working group telephone conference call that was held on July 8, 1993. The working group proposed two categories of protocols that may qualify for the accelerated review process: (1) multiple site protocols under the umbrella of the original RAC-approved protocol. Under such protocols, the "original" PI will have full responsibility for quality control and data reporting at satellite sites, and (2) protocols that are similar to other RAC-approved protocols; however, minor modifications have been introduced that do not involve gene transfer aspects of the studies. He stated that a check list is being developed for use in screening protocols that may qualify for the accelerated review process.

One major issue that remained unresolved by the working group is the uncertainty about how to proceed with the issue of Informed Consent documents that are different from those that were previously approved by the RAC. Dr. Parkman noted that Dr. Bruce Merchant of Viagene, San Diego, California, submitted a letter dated August 12, 1993,

requesting that the RAC consider certain categories of vaccine protocols as "exempt" from the *NIH Guidelines* and, therefore, exempt from RAC review.

Discussion

Dr. Zallen said that it is not practical to have a standardized Informed Consent document for all different institutions and protocols. Instead, she suggested 6 essential elements that should be considered when developing the Informed Consent documents: (1) recommendations about the use of birth control, (2) financial costs to patients, (3) the necessity for long-term follow up, (4) a request for autopsy, (5) timing of press releases relating to research, and (6) how patient confidentiality will be maintained.

In her written comments, Ms. Meyers stated that it is premature for any gene transfer protocols to proceed without prior RAC review. The number of patients treated to date is relatively small, and the issues of long-term safety and clinical benefit of gene transfer are largely unresolved.

Dr. Post agreed with the working group's recommendations and suggested an additional three categories of protocols that could qualify for exemption from RAC review: (1) vaccine protocols, including those using vaccinia viruses, adenoviruses and retroviruses, (2) those in which transduced cells (using a RAC-approved vector) are lethally irradiated prior to administration to human subjects, and (3) gene marking protocols with RAC-approved vectors.

Dr. Straus noted that the *NIH Guidelines* definition of "vaccines" as described in footnote 21 is unclear. Footnote 21 states that:

> "Section III-A-4 covers only those experiments in which the intent is to modify stably the genome of cells of a human subject. Other experiments involving recombinant DNA in human subjects such as feeding of bacteria containing recombinant DNA or the administration of vaccines containing recombinant DNA are not covered in Section III-A-4 of the Guidelines."

Dr. Straus explained that although vaccinia viruses do not persist in the host, other viruses such as herpes viruses, adenoviruses, papovaviruses, and retroviruses persist either through integration into the host genome, or stably within host cells. He suggested that a distinction should be made as to the categories of vaccines that are considered exempt from the *NIH Guidelines* and RAC review.

Ms. Buc asked the RAC if it might consider relinquishing its purview over human gene transfer protocols entirely. Mr. Capron explained that the RAC should not relinquish its responsibilities since there are many ethical questions for the RAC to consider, i.e., germ-line intervention. Dr. Chase added that the RAC serves to inform the public about broad issues involving recombinant DNA research in an unbiased and scientifically informed manner. Dr. Miller noted that the Food and Drug Administration's (FDA's) review of protocols is not held in a public format; and at this stage of gene transfer

research, public review by the RAC is very important.

Dr. Parkman said that the RAC review of individual protocols is essential to the development of science policy concerning gene therapy. Dr. Walters commented that the first cystic fibrosis (CF) protocol reviewed by the RAC in December 1992 is an example that illustrates the importance of public deliberation by the RAC. Not only did the RAC consider a new disease for the first time, but also a new vector (adenovirus). The public discussion of the CF protocols was very productive and useful in establishing policy for the review of other protocols.

There was a lengthy discussion about the interpretation of the Footnote 21 of the *NIH Guidelines* in regard to the types of vaccine protocols that are considered exempt. Dr. Merchant stated that although submission of protocols for RAC review is on a voluntary basis for commercial industry not associated with NIH-funded investigators, it is not realistic to conduct serious commercial development efforts without engaging NIH-funded investigators or institutions. For commercial research development, the time required to complete the review process is critical in order to bring the product to market. An exempt or accelerated review process would facilitate the process.

Dr. Walters requested that Drs. Parkman, Post, Carmen, and Straus form a Working Group on Categories for Accelerated Review to report back later on in the meeting. The RAC tabled their discussion until the next day to allow the working group to develop a revised list of possible categories of accelerated review protocols.

X. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *PILOT STUDY OF TOXICITY OF IMMUNIZATION OF PATIENTS WITH UNRESECTABLE MELANOMA WITH INTERLEUKIN-2 SECRETING ALLOGENEIC HUMAN MELANOMA CELLS*/ DRS. DAS GUPTA AND COHEN

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol resubmitted by Drs. Tapas K. Das Gupta and Edward P. Cohen of the University of Illinois College of Medicine. This protocol was deferred at the June 1993 RAC meeting until the investigators could return to the full RAC with the following: (1) data demonstrating the efficiency of transduction in Mel-4 cells; (2) data demonstrating viability, IL-2 production, and *in vivo* effect of irradiated transduced cells (either 5,000 or 10,000 rads) in the murine model; (3) rationale for ethnic eligibility criteria; (4) complete responses to the *Points to Consider*; and (5) RCR testing data demonstrating safety of the vector preparation.

Dr. Smith stated that this study is a Phase I trial using a well characterized human melanoma cell line that is transduced with the human IL-2 gene as an immunogenic vaccine. Twelve patients with advanced stage melanoma will be accrued on the study. The IL-2 secreting melanoma cells will be administered to patients who differ in at least

3 out of 6 alleles from the melanoma cell line at the Class 1 major histocompatibility locus. The transduced cells will be lethally irradiated with 5,000 rads. The major endpoint of the study will be toxicity. Other minor endpoints include measurement of induction of B and T cell responses to tumor cells and any potential clinical anti-tumor effect. The investigators have addressed most of the previous concerns raised by the RAC. Additional data on the irradiation of the tumor cells and production of IL-2 have been provided. Further information has been provided regarding the efficacy of the therapy in the murine model. The Informed Consent document has been revised and addresses the issue of financial responsibility for research expenses and routine care. Results for detection of RCR have been provided. Dr. Smith recommended approval of the present protocol.

Review--Ms. Buc

Ms. Buc commented primarily on the Informed Consent document. She suggested several changes to avoid misleading patients that any efficacy will be expected from this Phase I toxicity study. She expressed uneasiness about a statement that participation in the study may be halted at the sole discretion of the PI should contraception be interrupted.

Other Comments

Ms. Meyers raised several questions in her written comments about financial responsibility and patient privacy that have been addressed by the investigators. Dr. Haselkorn's written comments requested identification of the investigator who will be responsible for conducting the clinical study since Dr. Richards, a co-investigator on the protocol, is no longer employed by the University of Chicago.

Dr. Miller asked about the adequacy of the experiment to test viability of the cells after irradiation. The number of cells tested were far below that to be administered to patients. He questioned adequacy of the RCR assays for detecting viruses that might be activated in the irradiated human cells.

Dr. Walters raised several concerns about the Informed Consent document. The request for post-mortem should not be stated as if agreement to this procedure is a condition for entering this study. The *Purpose of the Research Study* section should begin with a sentence that informs patients that this protocol is a toxicity study, and that no therapeutic benefit is expected.

Ms. Buc remarked that under the Uniform Anatomical Gift Act, patients have the right to agree to donate organs or tissues for medical studies. A lengthy discussion ensued regarding whether families can override the patient's wishes. Mr. Capron stated that a patient can legally donate his/her body for a scientific study over the objection of family members under the Uniform Anatomical Gift Act. Dr. Chase suggested that a joint Informed Consent process in which patient's assent and the relatives' intent for autopsy could be obtained simultaneously and would avoid later discord on this matter.

Investigator Response--Dr. Cohen

Dr. Miller stressed that the viability test on the irradiated cells should be performed using cell numbers that correlate to a patient dose, i.e., 10^8 cells. Dr. Cohen agreed to comply with this stipulation. Dr. Miller suggested RCR safety testing should be performed by co-cultivation with human target cells rather than mouse cells that may be resistant to infection by human RCR. The activated RCR can be assayed by a neo^R colony assay since it will package the vector with the neo^R gene. Dr. Cohen agreed to this suggestion.

In response to Dr. Haselkorn's question about Dr. Richards no longer being at the University of Chicago, Dr. Cohen stated that Dr. Richards has joined the faculty of the University of Illinois and will continue to collaborate on this project.

On the Informed Consent document issue, Dr. Cohen agreed to the RAC's recommendation about a request for autopsy.

Committee Motion

A motion was made by Dr. Smith and seconded by Ms. Buc to approve the protocol contingent on the review and approval of the following stipulations by the primary reviewers and by Dr. Miller: (1) submit data demonstrating that the proposed dose of radiation effectively inhibits viability using the number of cells proposed for a single patient dose, (2) submit colony assay data demonstrating lack of RCR using irradiated transduced melanoma cells, and (3) submit revised language for the Informed Consent document that explains how consent for autopsy will be obtained, i.e., invoking the Uniform Anatomical Gift Act or requesting assent from the patient's relatives. The motion passed by a vote of 17 in favor, 0 opposed, and no abstentions.

XI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *A PHASE I CLINICAL TRIAL TO EVALUATE THE SAFETY AND EFFECTS IN HIV-1 INFECTED HUMANS OF AUTOLOGOUS LYMPHOCYTES TRANSDUCED WITH A RIBOZYME THAT CLEAVES HIV-1 RNA*/DRS. WONG-STAAL AND POESCHLA

Review--Dr. Straus

Dr. Walters called on Dr. Straus to present his primary review of the protocol submitted by Drs. Flossie Wong-Staal, Eric Poeschla, and David Looney of the University of California, San Diego, La Jolla, California. This protocol is a Phase I study of a retrovirus construct containing a ribozyme insert that when expressed is capable of cleaving HIV transcripts and diminishes virus replication and spread. Ribozymes are potentially therapeutic RNA molecules that contain antisense sequences for specific recognition and RNA-cleaving enzymatic activity. The investigators have demonstrated that human T cell lines and primary peripheral blood T cells transduced with the hairpin ribozyme that cleaves HIV-1 RNA in the 5' leader sequence are intracellularly

immunized against challenge with HIV. The ribozyme acts at two steps in the viral life cycle by cleaving: (1) afferent genomic viral RNA, and (2) efferent viral mRNA expressed from integrated provirus. The investigators propose to evaluate the safety and efficacy of gene transfer in 6 patients with HIV-1 infection by reinfusing autologous CD4(+) T cells that have been transduced *ex vivo* with a retrovirus vector that expresses the hairpin ribozyme. The *in vivo* kinetics and survival of ribozyme-transduced cells will be compared by limiting dilution polymerase chain reaction (PCR) with cells transduced with a control vector. Dr. Straus stated that the PI is a renowned and accomplished retrovirologist, who has enlisted qualified clinical collaborators to facilitate the project. The protocol relies on a vector and a packaging cell line that are well defined. Dr. Straus raised the following concerns about the protocol: (1) What assays will be performed for the detection of RCR and who will perform these assays? (2) Is rabbit pyrogen testing of clinical materials necessary? (3) Is azidothymidine (AZT) antiviral therapy required for subjects entering this study? and (4) Will 4 or 6 patients be accrued onto the study? Dr. Straus said that this study is creative and with clarification of these issues, he recommends approval of the protocol.

Review--Dr. Hirano

Dr. Hirano stated that the results of the preclinical studies and the objective of this protocol are very exciting, but there are five concerns that should be addressed: (1) Although the vector and packaging cell lines have previously been approved, production of vector supernatant is currently being performed in a different laboratory. Results of RCR testing in the new laboratory should be provided. (2) There is a lack of PCR data comparing the survival of transduced T cells in the background of a large excess of untransduced cells as a function of time in tissue culture. (3) Transduction efficiency and the ability to expand these transduced cells to numbers that correlate to a patient dose has not been demonstrated. (4) The transduction procedure described in the preclinical studies is different from that proposed in this protocol. (5) Data are not provided regarding testing of ribozyme toxicity *in vivo* in the severe combined immunodeficiency (SCID)-Hu mouse model. (6) The effectiveness of the ribozyme in cleaving HIV RNA in lymphocytes obtained from HIV patients with uncloned virus strains has not been demonstrated.

Review--Dr. Chase

Dr. Chase raised four major issues: (1) The investigators are proposing to use FDA criteria for RCR testings, which have not been totally agreed upon by the RAC. (2) The necessity for AZT administration, which was mandated by IRB, is questionable. Dr. Chase recommended that AZT administration should be at the discretion of the treating physicians. (3) The financial risks to patients for treatment procedures are not clearly stated in the Informed Consent document. (4) The kinetics of cell survival, which compares ribozyme-transduced with control cells, have not been adequately described. Pending clarification on these issues, Dr. Chase recommended approval of the protocol.

Other Comments

Ms. Meyers indicated several concerns in her written comments about the Informed Consent document language. The financial responsibility of patients is not clearly stated. The request for autopsy includes a statement that the patient's permission for autopsy may not be rescinded by relatives. Mr. Capron said that he would defer to the opinion of the legal counsel of the University of California to determine whether this language is enforceable without invoking the Uniform Anatomical Gift Act.

Dr. Carmen suggested several specific language changes in the Informed Consent document to convey more clearly the recombinant DNA aspects of this protocol to patients. Dr. Smith expressed concern about the ambiguous statements of financial risks to patients and the lack of request for autopsy in the Informed Consent document. He stated that the IRB stipulation of mandatory AZT is unnecessary. Also, there is a question about the possibility of generating RCR with an altered host range by administering a retrovirus vector to HIV infected patients.

Regarding the issue of AZT use in acquired immunodeficiency syndrome (AIDS) patients, Dr. Straus said that there is a changing opinion in the medical community resulting from new studies. In patients with CD4 counts below 200, AZT appears to have beneficial effect on patient survival. But for patients with CD4 counts between 250 and 600, which is the target patient population of this study, the current data are conflicting as to the beneficial effect of AZT. Dr. Straus was concerned about the mandatory use of AZT as suggested by the IRB. The decision to use antiviral drugs is best left to the primary care physicians. Dr. Post also expressed his concern about the requirement for AZT in healthy HIV(+) individuals.

Dr. Walters requested a clearer statement in the Informed Consent document as to whether there will be any clinical benefit to patients in this Phase I trial. Mr. Braverman commented on the proposed study. He expressed his reservation that the IRB has mandated the use of AZT for patients in this trial. He raised several points regarding inclusion and exclusion criteria for patients in this trial. Overall, he was pleased to see this gene therapy protocol reviewed by the RAC and applauded such an advancement in AIDS research. Dr. Nava Sarver, National Institute of Allergy and Infectious Diseases, NIH, expressed her support for this protocol to evaluate ribozyme treatment in HIV(+) individuals.

Investigator Response--Drs. Wong-Staal and Poeschla

Regarding the question of RCR testing, Dr. Wong-Staal said that they are in the process of seeking an established laboratory to test clinical grade preparations. Preliminary results from her own laboratory indicate no RCR contamination. Responding to questions raised by Drs. Hirano and Chase regarding the detection of ribozyme transduced cells in the presence of untransduced cells, Dr. Wong-Staal presented data demonstrating that the PCR assay is capable of detecting at least one transduced cell in the background of 5,000 untransduced cells. Dr. Chase pointed out that the real question is how to analyze the differential survival rates of ribozyme transduced versus untransduced cells in HIV patients, and this question is not adequately addressed by this

experiment. As to transduction efficiency of the retroviral vector, Dr. Wong-Staal said the initial transduction rate is only a few percent. However, after G418 selection, the ribozyme-transduced T cells are resistant to challenge by two HIV isolates. Dr. Parkman commented that other investigators have encountered some technical difficulties in performing G418 selection of transduced cells obtained from patient samples due to non-specific toxicity to lymphocytes, particularly, in the scaling up of this procedure to obtain large quantity of cells for patient use. Dr. Wong-Staal agreed that a rehearsal of this isolation procedure at the clinical scale will be performed. Dr. Poeschla addressed questions on the assays that will be performed to detect HIV in the transduced lymphocyte preparations that will be administered to patients. Any preparation positive in the p24 HIV test will be discarded.

Dr. Poeschla explained that there are differing opinions between the investigators and their IRB regarding the mandatory requirement for AZT administration. Not only is there controversy about the use of AZT, but the IRB has mistakenly assumed that AZT is useful for inhibiting murine RCR in the event of RCR contamination (AZT was used in Dr. Nabel's previously approved HIV protocol). Mr. Capron suggested that if there is a misunderstanding on the part of the IRB, the RAC should clarify the issue. Dr. Parkman agreed that the risk/benefit ratio argues against mandatory AZT treatment for patients who do not need the drug. Dr. Poeschla stated that the rabbit pyrogen test required by his IRB is neither sensitive nor specific. Although the activated lymphocytes may produce cytokines that are pyrogenic, the fever experienced by patients can be readily treated with medication. Regarding how the number of patients was selected, Dr. Poeschla said that is not critical because this is a Phase I trial to compare survival of lymphocytes within the same individual.

Regarding questions about the Informed Consent document, Dr. Poeschla agreed to the changes suggested by Dr. Carmen. As to the directive made by patients to consent for autopsy irrespective of a relative's objection, Dr. Poeschla said that this language is requested by most of his HIV(+) patients. In regard to the question of any potential benefit to the patients, Dr. Poeschla said that considering the small number of cells to be administered to patients, the clinical benefit is very small, and it will be stated clearly in the Informed Consent that there will be no clinical benefit. In future trials, the use of stem cells may have a greater potential to affect disease progression.

Dr. Hirano stated that a rehearsal scale-up experiment must be performed to ensure that lymphocytes obtained from HIV(+) patients are transduced with the vector, enriched by G418 selection, checked for lack of HIV, and grown up to clinical quantity.

Dr. Straus suggested that a letter be forwarded from ORDA to the IRB recommending that AZT administration and rabbit pyrogen testing should not be mandatory.

Committee Motion

A motion was made by Dr. Straus and seconded by Dr. Chase to approve the protocol contingent on the following: (1) a letter will be forwarded from ORDA to the IRB

recommending that AZT administration and rabbit pyrogen testing should not be mandatory, (2) the RAC will be notified of the name of the laboratory that will conduct RCR testing on the master cell bank and a detailed list of the tests that will be performed, and (3) the investigators will demonstrate that they can perform the transduction procedure on a clinical scale using cells from HIV-infected patients. The motion passed by a vote of 17 in favor, 0 opposed and 1 abstention (Dr. Geiduschek abstained from voting because he is employed by the same institution).

XII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE THERAPY PROTOCOL ENTITLED: *GENETICALLY ENGINEERED AUTOLOGOUS TUMOR VACCINES PRODUCING INTERLEUKIN-2 FOR THE TREATMENT OF METASTATIC MELANOMA*/DRS. ECONOMOU AND GLASPY

Review--Dr. Motulsky

Dr. Walters called on Dr. Motulsky to present his primary review of the protocol submitted by Drs. James S. Economou and John Glaspy of the University of California, Los Angeles, California. This protocol is a study of patients with metastatic melanoma who have failed standard therapy. In an attempt to increase the patient's immune response to the tumor, the IL-2 gene will be introduced into a human melanoma cell line (M-24). The gene-modified melanoma cell line producing IL-2 will then be mixed with tumor cells obtained from the patient. This mixture of cells will be lethally irradiated and injected subcutaneously into patients. This injection is expected to augment the immune response of the patients to tumor cells through the immune stimulatory effects of IL-2 secreted by the gene-modified cells. Animal models have shown that injection of gene-modified cells has important anti-tumor effects. To determine the safety of the procedure, a constant number of tumor cells will be mixed with escalating numbers of IL-2 producing cells. The study will involve 30 patients in 3 dose-escalation groups. The following endpoints will be measured: (1) toxicity, (2) generation of CTL precursors, lymphokine activated killer (LAK) cells and enhanced natural killer (NK) activity, (3) immunohistochemistry and delayed-type hypersensitivity skin tests using injection of irradiated autologous tumor cells, and (4) clinical anti-tumor responses. Dr. Motulsky stated that most of his initial concerns have been adequately addressed by the investigators. Several questions remain: Have experiments been performed in which IL-2 is injected locally to observe any effect on tumor growth? What is the rationale of mixing the allogeneic M24 cells in the vaccine cocktail? Would other IL-2 producing cells, such as transduced B-lymphocytes, serve the same purpose? Will HLA typing of autologous and allogeneic cells be performed?

Dr. Motulsky provided the following comments in regard to the Informed Consent document: (1) abbreviations should be avoided since they may not be understood by laypersons, (2) the use of the term "vaccine" is inappropriate and misleading, (3) the statement that describes the patient's consent as "received" should be more appropriately be described as "given", (4) the issue of financial risk to patients for costs associated with an investigational procedure should be addressed, and (5) the statement that addresses the commercial value of cell lines should be clarified. The investigators should provide

the RAC with information about the source of the proposed cell line. In conclusion, the study may demonstrate the potential to reduce human tumor growth, and there do not appear to be any new problems associated with the gene transfer portion of the proposal.

Review--Dr. Doi

Dr. Doi stated that the investigators have responded to most of his initial concerns; however, he noted that the use of the term "vaccine" in the title, throughout the protocol, and in the Informed Consent documents is misleading to patients and others. The proposed material to be used in the treatment is not a "vaccine" in the classical sense. The investigators should respond to the following questions: (1) How many transduced M24 cells are required to produce between 10^3 and 10^5 picograms of IL-2? (2) Are transduced cells stable during cryopreservation? (3) Will patients react immunologically to the allogeneic melanoma cell line after biweekly and monthly injections over a period of one year? Dr. Doi said that the overall experimental design appears to be based on sound and reasonable procedures, the study is straightforward, and the endpoints are well defined. Dr. Doi recommended approval of the protocol.

Review--Dr. Zallen

Dr. Zallen explained that her questions are directed to three areas. In regard to the animal and preclinical studies, most of the preclinical studies were performed with a different melanoma cell line, M14. Very little data was submitted using the M24 cell line that will be used for the clinical trial. The relevant animal studies described in a preprint have not been included as part of the proposal. The expected bystander effect has not been defined in the animal studies. In the area of experimental design and patient selection, HLA typing should be performed prospectively to confirm that there is a match with the M24 cell line before patients are enrolled onto the study. In most preclinical studies, cells were irradiated with 10,000 rads, yet 5,000 rads is proposed for the clinical study. Is 5,000 rads a sufficient dose to lethally irradiate the M24 cells?

In regard to the Informed Consent process, Dr. Zallen asked the investigators to elaborate on how patient consent will be obtained. Several specific changes were suggested to the Informed Consent document: (1) the use of the term "vaccine" is misleading to the patients, (2) it is unclear as to the number of biopsies that will be performed and the associated risks, (3) the section that describes financial risks to patients that could result from research-related injuries should be clarified, and (4) an exaggerated number of $100,000 was originally assessed as the patient's upper limit of financial costs. That figure should be removed from the Informed Consent document.

Other Comments

Dr. Smith asked whether there are data demonstrating that 5,000 rads is sufficient to lethally irradiate the M24 cells. Dr. Parkman said that HLA typing of patients will facilitate interpretation of research results because differences in patient responses between those who share HLA antigens with the M24 melanoma cell and those who do

not will be interpretable. Dr. Parkman asked the investigators to summarize their earlier results from studies using the M24 cell with and without systemic IL-2 administration. Dr. Geiduschek asked whether the IL-2 producing M24 cells will be derived from a clonal cell line or mass cell culture transduced by the vector? Will the cell line be selected for high IL-2 secretion?

Ms. Meyers raised concerns in her written comments about the use of the term "vaccine" and the statement about the financial costs for patients participating in this trial. The Informed Consent document does not include a request for autopsy. Dr. Walters questioned if the first sentence of the Informed Consent document adequately states the purpose of the study.

Investigator Response--Dr. Economou

Dr. Economou explained that the present study combines the merit of two approaches of immunotherapy of cancer, i.e., transduction of an IL-2 expressing vector in patients' autologous tumor cells and an allogeneic cell line. Studies in animal models suggest that a "bystander effect" is produced by this immunization. Reduced tumorigenicity and enhanced immunogenicity is achieved by mixing untransduced tumor cells with immunologically irrelevant cytokine-producing cells. The local continuous production of cytokines by cells adjacent to autologous tumor cells is sufficient to provoke immunological responses to tumors. In regard to the rationale for selecting the M24 melanoma cell line for transduction by the IL-2 vector, he explained that the IRB urged him to use M24 because it is a well characterized cell line that has been used extensively in previous clinical trials, and it appears to be safe for human use.

Clarifying the question about the source of funding for the present study, Dr. Economou said that the present study will be funded by NIH through an implementation program project grant. Genetic Therapy, Inc., will provide scientific collaboration but no direct financial support. The IL-2 producing cell line will be made in the investigator's laboratory that is funded by NIH.

Responding to a question about lack of direct data on M24 cells, Dr. Economou said that in preclinical studies the M14 cell line was used, and similar results were obtained with the M24 cell line although those data were not included in their published paper. Dr. Zallen said those relevant data should be presented in this proposal. Dr. Miller asked whether the investigators have already isolated a high IL-2 producing cell line. Dr. Economou responded that they have not isolated a high producer to date. The investigators are waiting for the biosafety testing requirements from FDA. As to the question of whether 5,000 rads irradiation is sufficient to inhibit cell proliferation, Dr. Economou said that 5,000 rads is the dose used for this cell line in other tumor vaccine studies at the University of California, Los Angeles. No tumor growth has ever been observed at the inoculation site in other clinical trials. Responding to Dr. Doi's question about possible adverse effects from repeated immunization with M24 cells, Dr. Economou said that no adverse immunological reaction has been observed in the last 10 years of clinical trials with these cell lines. Dr. Economou agreed to perform HLA

typing prior to entering patients onto the study. There was a lengthy discussion about the scientific justification for approving this type of clinical immunotherapy trial, i.e., using cytokine producing cells and autologous tumor cells. Several RAC members expressed their reservation about the lack of any definitive result from other RAC-approved clinical trials that were similar to this proposal.

Committee Motion

A motion was made by Dr. Motulsky and seconded by Dr. Doi to approve the protocol contingent on review and approval of the following stipulations: (1) submission of transduction data on the M24 cell line, (2) an indication of the PI's intent to perform HLA typing prior to enrolling the patients onto the study, and (3) submission of data demonstrating the biological activity of IL-2 of transduced M24 cells in the animal model. The motion passed by a vote of 11 in favor, 0 opposed, and 6 abstentions.

XIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING SEMLIKI FOREST VIRUS (SFV) VECTOR EXPRESSION SYSTEM--REDUCTION IN PHYSICAL CONTAINMENT FROM BL3 TO BL2/DR. TEMPLE

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the proposal resubmitted by Dr. Gary F. Temple of Life Technologies, Inc., Gaithersburg, Maryland. This request was deferred at the September 14, 1992, and June 8, 1993, RAC meetings. The proposal was deferred during the September 1992 RAC meeting until the investigators return to the full RAC with data regarding the following: (1) the frequency of recombination that produces replication-competent virus, using cell numbers analogous to the laboratory setting (e.g., 1×10^9 cells), and (2) acquire data regarding the frequency of seropositivity among personnel previously exposed to SFV. The proposal was deferred during the June 1993 RAC meeting until the investigators could return to the full RAC with the following: (1) a product information sheet informing customers of the potential health risk of the expression system, standard methods to be used for virus inactivation, a helper virus assay to detect SFV, and a description of symptoms and procedures to be followed in the event that SFV infection occurs in a laboratory worker (including methods to prevent transfer to insect vectors and environmental spread); and (2) SFV inactivation data.

Dr. Miller stated that the investigators are requesting a reduction in the physical containment level from Biosafety Level (BL) 3 to BL2 for Life Technologies' SFV/Helper 2 Gene Expression System. The investigators have produced a Users' Manual as recommended by the RAC. This manual contains the following information: (1) a summary of possible health risks associated with the use of the cloning system, (2) a description of practical and effective means to inactivate SFV, (3) a simple protocol for the detection of replication-competent SFV, (4) a description of symptoms associated with SFV infection and procedures to be followed in the event of laboratory infection,

(5) warnings applicable to BL2 guidelines, (6) a "Biosafety Clearance Agreement" to be signed by the user and his/her IBC, and (7) a listing of BL2 practices advisable for use with the cloning system. Dr. Miller commended the investigators for the Users' Manual and stated that the investigators have adequately responded to all of the RAC's previous concerns. Dr. Miller recommended approval of the request to reduce the physical containment level from BL3 to BL2.

Review--Dr. Krogstad

Dr. Krogstad commented on the well prepared Users' Manual. This manual addresses most of the concerns raised during the previous RAC review. He suggested that the important areas of the manual should be highlighted in boldface type. The only issue still of concern is how laboratory personnel will be informed of the potential risks. Every individual in the laboratory, even if they do not use the SFV system, should sign the "Biosafety Clearance Agreement" as an acknowledgement that they have been informed of the potential risks associated with the SFV expression system. SFV is an infectious agent that can spread by aerosol. He recommended reducing only the containment level for the expression system from BL3 to BL2; however, for the parent virus, SFV, which is a Class 3 agent, the containment level should not be reduced. There is still substantial risk of serious disease among people who become infected as a result of laboratory accidents or as a result of natural transmission.

Review--Dr. Post

Dr. Post stated that the Users' Manual prepared by the investigators addresses all the previous concerns raised by the RAC, and he recommended use of this SFV/Helper 2 Gene Expression System at BL2 containment. Dr. Post still expressed his concern about the discrepancy between the classification of Sindbis virus (Class 2) and SFV (Class 3). The scientific justification for this distinction is weak.

Other Comments

Dr. Miller said that requiring all laboratory personnel to sign the Biosafety Clearance Agreement will be unworkable. It should be the PI's responsibility to ensure that all laboratory personnel have been adequately informed. Dr. Geiduschek and Dr. Straus considered that it is appropriate for all individuals who are likely to be exposed to this agent to acknowledge the risks in writing.

Investigator Response--Dr. Temple

Dr. Temple stated that in compliance with the *NIH Guidelines*, everyone entering a BL2 facility must be informed of the activities within the laboratory. The PI is responsible for ensuring compliance with the *NIH Guidelines*. For practical reasons, it is difficult to keep track of all individuals who are entering and exiting the facility. Dr. Temple explained that according to the *NIH Guidelines*, when an infectious agent is in use in a laboratory, there are special entry requirements, i.e., a hazard warning sign must be

posted, and the access to the laboratory is limited to those individuals authorized by the PI. In cases of mixed-use facilities, the containment requirement for the highest level of activities should be observed by all individuals within the same facility.

Dr. Straus expressed his concern about commercial distribution of the SFV/Helper 2 Gene Expression System even if all the BL2 safety rules are observed. He said he is still concerned about the pathogenicity of SFV, noting that an incidence of fatality was previously reported. The rationale for safe use of this agent in the laboratory is based on experiences of a very limited number of individuals (approximately 50). This sample size of individuals is too small to predict risk for the wider use that would occur if this product becomes commercially available. An example of this risk is polio virus which causes paralysis in 1 out of 1,000 infected individuals. Although it has been handled safely by qualified investigators in the laboratory, its use as a cloning vector would not be justified in an unvaccinated population. Dr. Temple said that over 400 laboratory workers have successfully worked with SFV over the last decade, and there have been no instances of symptomatic disease.

Dr. Miller agreed with Dr. Krogstad that a stipulation for approval should be added that would require that the PI obtain signatures from all laboratory personnel who are regularly present in the laboratory and agreed that this stipulation adequately addresses the safety concern.

Committee Motion

A motion was made by Dr. Miller and seconded by Dr. Krogstad to approve the request to reduce the level of physical containment for SFV/Helper 2 Gene Expression System to be distributed by Life Technologies, Inc., from BL3 to BL2. Approval of the request is contingent on the requirement that the PI must obtain signatures from all laboratory personnel certifying that they have been informed of the possible risks associated with this expression system and that they have read the Users Manual. The motion to approve the request passed by a vote of 13 in favor, 2 opposed, and 1 abstention.

XIV. AMENDMENT TO APPENDIX B OF THE *NIH GUIDELINES* REGARDING UPDATING APPENDIX B: CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD/DR. FLEMING

Presentation--Dr. Fleming

Dr. Walters called on Dr. Fleming of the Mid-Atlantic Biological Safety Association, Bowie, Maryland, to present her updated listing of Appendix B: Classification of Microorganisms on the Basis of Hazard. Dr. Fleming explained that the current Appendix B is based on biohazard information about etiologic agents obtained in the 1970s. Since that time, there have been several updated publications from the Centers for Disease Control (CDC) and the NIH. An updated classification was published in 1980, and was incorporated into a book entitled: *Biosafety in Microbiological and Biomedical Laboratories*, published in 1984. The latest edition of the NIH/CDC

publication was published in May 1993 and is available from the Government Printing Office. The NIH/CDC publication contains quantitative risk-assessment information; however, this information has not been developed into a simplified list, i.e., updated Appendix B. The proposed Appendix B has been compiled from information derived from the NIH/CDC publication. Dr. Fleming stated that a copy of proposed Appendix B has been forwarded to the CDC for review.

Discussion

Dr. Miller noted that SFV is listed as both a Class II and a Class III agent, and it is unclear which containment level should be used. Dr. Post expressed concern about the interpretation of the information in the CDC/NIH publication and how this information has been transformed into the proposed Appendix B. Dr. Post stated that the RAC does not have the expertise or time to assess the accuracy of this interpretation. Many RAC members expressed similar lack of expertise to properly assess the accuracy of this list, and deferred voting on this proposal until expert opinions have been obtained from the CDC and NIH Division of Safety.

Committee Motion

The RAC recommended by consensus that the current classification of etiologic agents described in *Biosafety in Microbiological and Biomedical Laboratories*, 3rd edition, May 1993, U. S. Department of Health and Human Services, should be endorsed by the RAC; however, the RAC retains the option to adopt any modifications to the CDC listing. The RAC recommended that the summary list (proposed Appendix B) should not be adopted by the RAC until the subcommittee receives letters of concurrence from both the CDC and NIH Division of Safety.

XV. AMENDMENTS TO SECTIONS III, IV, V AND APPENDIX C AND F OF THE *NIH GUIDELINES* REGARDING THE CLONING OF TOXIN MOLECULES/DR. WIVEL

Dr. Walters called on Dr. Wivel to present his request for amendments to Sections III, IV, and V, and Appendices C and F regarding the review process for experiments involving the cloning of toxin molecules. Dr. Wivel stated that these amendments will establish a new category of review entitled: Experiments that Require NIH (ORDA) and IBC Approval Before Initiation. Under this new category of review, experiments involving the cloning of toxin molecules that are lethal for vertebrates at an LD_{50} of < 100 nanograms per kilogram of body weight will be reviewed by NIH (ORDA) in consultation with *ad hoc* toxin experts. Sections III, IV, V and Appendix C and F are proposed to read:

> "Section III. Guidelines for Covered Experiments.
>
> "Part III discusses experiments involving recombinant DNA. These experiments have been divided into five classes:

"III-A. Experiments which require specific RAC review and NIH and IBC approval before initiation of the experiment;

"III-B. Experiments which require NIH (Office of Recombinant DNA Activities/ORDA) and Institutional Biosafety Committee (IBC) approval before initiation of the experiment;

"III-C. Experiments which require IBC approval before initiation of the experiment;

"III-D. Experiments which require IBC notification at the time of the experiment;

"III-E. Experiments which are exempt from the procedures of the Guidelines.

"IF AN EXPERIMENT FALLS INTO BOTH CLASS III-A AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS III-A MUST BE FOLLOWED. If an experiment falls into Class III-E and into either Class III-C or III-D as well, it can be considered exempt from the requirements of the Guidelines. Changes...."

Section III-A-1 will be moved to a new Section III-B-1. New Section III-B is proposed to read:

"Section III-B-Experiments That Require NIH (ORDA) and IBC Approval Before Initiation.

"Section III-B-1. Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight....

"Section III-B-1-(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH through ORDA. The containment conditions for such experiments will be determined by ORDA in consultation with *ad hoc* experts. Such experiments also require the approval of the IBC before initiation (see Section IV-C-1-b-(3)-(f)."

Sections III-A-2, III-A-3, III-A-4 will be renumbered to III-A-1, III-A-2, III-A-3 respectively. Sections III-B, III-C, and III-D will be renumbered to III-C, III-D, and III-E respectively.

The new Section III-C-2 is proposed to read:

"Section III-C-2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) is Cloned in Nonpathogenic

Prokaryotic or Lower Eukaryotic Host-Vector Systems.

"Section III-C-2-a. ...Many experiments in this category are exempt from the Guidelines (see Section III-E-4) and III-E-5). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH (ORDA) approval (see Section III-B-1) or must be carried out under NIH specified conditions as described in Appendix F."

Section IV-B-5-b-(3) is proposed to read:

"Section IV-B-5-b-(3). Petition NIH (ORDA), with concurrence of the IBC for approval to conduct experiments specified in Sections III-A and III-B of the Guidelines;"

Section IV-C-1-b-(3)-(f) will be deleted which reads: "Approving the cloning of toxin genes in host-vector systems other than *E. coli* K-12 (see Appendix F); and]"

Section IV-C-1-b-(3)-(g) will become the new Section IV-C-1-b-(3)-(f).

The new Section IV-C-3-a is proposed to read:

"Reviewing and approving experiments involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD_{50} ≤100 nanograms per kilogram body weight in organisms other than *E. coli* K-12 (see Section III-B-1 and Appendices F-I and F-II)."

Sections IV-C-3-a and IV-C-3-b will be renumbered to become Sections IV-C-3-b and IV-C-3-c respectively.

Section V-2 is proposed to read:

"...In the cases falling under Sections III-A through III-D, this judgment is to be reviewed and approved by the IBC..."

Appendix C is proposed to read:

"Appendix C. Exemptions Under Section III-D-5.

"...Appendix C-I. Recombinant DNA in Tissue Culture...

"...Exceptions. The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents, and (iv) experiments involving the cloning of toxin

molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-II. Experiments Involving *E. coli* K-12 Host-Vector Systems...

"...Exceptions. The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior IBC review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-III. Experiments Involving *Saccharomyces* Host-Vector Systems...

"...Exceptions. The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-IV. Experiments Involving *Bacillus subtilis* Host-Vector Systems...

"...Exceptions. The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F).

"...Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms...

"...Exceptions. The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III-B which require NIH (ORDA) and IBC approval before initiation, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes in *E. coli* K-12 (see Appendix F.)"

Appendix F is proposed to read:

"Appendix F. Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates.

"Appendix F-I. General Information.

"Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of <100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, *Shigella dysenteriae* neurotoxin) are covered under Section III-B-1 of the Guidelines and require NIH (ORDA) and IBC approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxin molecules with an LD_{50} of < 100 micrograms and > 100 nanograms per kilogram body weight require registration with ORDA and IBC approval prior to initiating the experiments. A list of toxin molecules classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxin molecules not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with *ad hoc* experts, prior to inclusion of the molecules on the list (see Section IV-C-1-b-(2)-(e))...

"Appendix F-III. Containment Conditions for Cloning Toxin Molecule Genes in Organisms Other Than *E. coli* K-12.

"Requests involving the cloning of genes coding for molecules toxic for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight in host-vector systems other than *E. coli* K-12 will be evaluated by NIH (ORDA) in consultation with *ad hoc* toxin experts (see Sections III-B and IV-C-1-b-(3)-(f).

"Appendix F-IV. Specific Approvals.

"An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

Appendix F-IV-A through Appendix F-IV-K would be deleted. [A list of these specific approvals will be maintained in ORDA.]

Committee Motion

The RAC approved a motion made by Mr. Capron and seconded by Dr. Post to accept

the amendments to Sections III, IV, and V and Appendix C and F of the *NIH Guidelines*. The motion passed as proposed by a vote of 16 in favor, 0 opposed, and no abstentions.

XVI. AMENDMENTS TO SECTION III AND APPENDIX D OF THE *NIH GUIDELINES* REGARDING ACTIONS TAKEN UNDER THE GUIDELINES/DR. WIVEL

Dr. Walters called on Dr. Wivel to present his request for an amendment to Section III and Appendix D. Dr. Wivel stated that these amendments would eliminate the requirement for publication of Appendix D (Actions Taken Under the Guidelines) in the *Federal Register* and to allow distribution of these actions by the Office of Recombinant DNA Activities. Section III-A and Appendix D is proposed to read:

> "Section III-A--Experiments that Require RAC Review and IBC Approval Before Initiation.
>
> "...Specific experiments already approved in this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."
>
> "Appendix D--Actions Taken Under the Guidelines.
>
> "As noted in the subsection IV-C-1-b-(1), the Director, NIH, may take certain actions with regard to the Guidelines after the issues have been considered by the RAC. An updated list of these actions are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

Committee Motion

The RAC approved a motion made by Mr. Capron and seconded by Dr. Chase to accept the amendments to Section III and Appendix D of the *NIH Guidelines*. The motion passed as proposed by a vote of 16 in favor, 0 opposed, and no abstentions.

XVII. WORKING GROUP REPORT ON CATEGORIES OF HUMAN GENE TRANSFER EXPERIMENTS THAT ARE EXEMPT FROM RAC REVIEW/DR. PARKMAN

Dr. Walters called on Dr. Parkman, Chair of the Working Group on Categorization of Protocols, to summarize the conclusions of the working group. Dr. Parkman stated that the working group proposes three possible categories of experiments that may be eligible for accelerated review by the RAC: (1) protocols conducted at satellite institutions under the responsibility of the PI, (2) protocols that are identical to another NIH-approved human gene transfer protocol under the responsibility of a new PI, (3) protocols that are similar to NIH-approved protocols; however, minor changes have been introduced that do not affect the gene therapy aspects of the study, and (4) protocols that use lethally irradiated cells (the RAC did not reach a consensus about the kinds of vectors that would qualify).

Dr. Parkman proposed the following: (1) ORDA would assume the responsibility for making determinations whether protocols would qualify for accelerated review status and

qualifications of any new investigators, (2) a summary of approved accelerated review protocols would be presented to the full committee at scheduled RAC meetings, and (3) semi-annual data reports would be required for all accelerated review protocols in order to monitor for evidence of gene transfer and possible adverse effects.

Dr. Walters proposed that the working group continue to refine the criteria for accelerated review protocols and to develop draft language for inclusion in the *NIH Guidelines*. This material will be reviewed by the full RAC at its December 1993 meeting. The RAC will also reconsider the definition of recombinant DNA vaccines with the view that Footnote 21 in Section V of the *NIH Guidelines* may need to be revised.

XVIII. FUTURE MEETINGS OF THE RAC

Dr. Walters announced that the next meeting of the RAC will be December 2-3, 1993, NIH, Building 31C, Conference Room 6.

XIX. ADJOURNMENT

Dr. Walters adjourned the meeting at 3:30 p.m. on September 10, 1993.

Nelson A. Wivel, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Date: 12/2/93

LeRoy B. Walters, Ph.D.
Chair
Recombinant DNA Advisory Committee
National Institutes of Health

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8810-001* (M) Rosenberg, Steven A., National Cancer Institute; *The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2 and Tumor Infiltrating Lymphocytes.*
Date of RAC Approval: 10-3-88
Date of NIH Approval: 3-2-89
Minor Modification: 3-30-90

9007-002** (T) Blaese, R. Michael, National Cancer Institute; *Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells*
Date of RAC Approval: 7-31-90
Date of NIH Approval: 9-6-90
Major Amendment-Date of RAC Approval: 2-10-92
Major Amendment-Date of NIH Approval: 4-22-92
Minor Modification: 4-21-93
Minor Modification: 6-7-93

9007-003 (T) Rosenberg, Steven A., National Cancer Institute; *Gene Therapy of Patients with Advanced Cancer Using Tumor Infiltrating Lymphocytes Transduced with the Gene Coding for Tumor Necrosis Factor.*
Date of RAC Approval: 7-31-90
Date of NIH Approval: 9-6-90

9102-004* (M) Brenner, Malcolm K.; Mirro, Joseph; Hurwitz, Craig; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital; *Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
Date of RAC Approval: 2-4-91
Date of NIH Approval: 7-12-91
Minor Modification: 11-91

9105-005* (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital; *A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
Date of RAC Approval: 5-31-91
Date of NIH Approval: 7-12-91
Minor Modification: 11-91

9105-006* (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital; *A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement.*
Date of RAC Approval: 5-31-91
Date of NIH Approval: 7-12-91
Minor Modification: 11-91

9105-007* (M) Deisseroth, Albert B., M.D. Anderson Cancer Center; *Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial.*
Date of RAC Approval: 5-31-91
Date of NIH Approval: 7-12-91
Minor Modification: 4-19-93

9105-008 (M) Ledley, Fred D.; Woo, Savio; Ferry, George; and Hartwell, Whigennand, Baylor College of Medicine; *Hepatocellular Transplantation in Acute Hepatic Failure and Targeting Genetic Markers to Hepatic Cells.*
Date of RAC Approval: 5-30-91
Date of NIH Approval: 7-12-91

9105-009* (M) Lotze, Michael T., University of Pittsburgh School of Medicine; *The Administration of Interleukin-2 and Tumor Infiltrating Lymphocytes to Patients with Melanoma.*
Date of RAC Approval: 5-30-91
Date of NIH Approval: 1-17-92
Minor Modification: 11-30-92

9110-010* (T) Rosenberg, Steven A., National Cancer Institute; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF).*
Date of RAC Approval: 10-7-91
Date of NIH Approval: 10-15-91
Minor Modification: 7-9-92

9110-011 (T) Rosenberg, Steven A., National Cancer Institute; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Interleukin-2 (IL-2).*
Date of RAC Approval: 10-7-91
Date of NIH Approval: 10-15-91

9110-012* (T) Wilson, James M., University of Michigan; *Ex Vivo Gene Therapy of Familial Hypercholesterolemia.*
Date of RAC Approval: 10-8-91
Date of NIH Approval: 11-14-91
Minor Modification: 12-3-92
Minor Modification: 8-18-93

9202-013* (T) Nabel, Gary J., University of Michigan; *Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors.*
Date of RAC Approval: 2-10-92
Date of NIH Approval: 4-17-92
Minor Modification: 1-22-93
Minor Modification: 1-93

9202-014 (M) Cornetta, Kenneth, Indiana University; *Retroviral-Mediated Gene Transfer of Bone Marrow Cells during Autologous Bone Marrow Transplantation for Acute Leukemia.*
Date of RAC Approval: 2-11-92
Date of NIH Approval: 4-17-92

9202-015 (M) Economou, James S. and Belldegrun, Arie, University of California at Los Angeles; *The Treatment of Patients with Metastatic Melanoma and Renal Cell Cancer Using In Vitro Expanded and Genetically-Engineered (Neomycin Phosphotransferase) Bulk, CD8 (+) and/or CD4(+) Tumor Infiltrating Lymphocytes and Bulk, CD8(+) and/or CD4(+) Peripheral Blood Leukocytes in Combination with Recombinant Interleukin-2 Alone, or with Recombinant Interleukin-2 and Recombinant Alpha Interferon.*
Date of RAC Approval: 2-11-92
Date of NIH Approval: 4-17-92

9202-016* (T) Freeman, Scott M., University of Rochester School of Medicine, Rochester, New York; *Gene Transfer for the Treatment of Cancer.*
Date of RAC Approval: 2-10-92
Date of NIH Approval: 2-5-93
Minor Modification: 8-6-93

9202-017 (M) Greenberg, Philip D. and Riddell, Stanley, University of Washington, Seattle; *Phase I Study of Cellular Adoptive immunotherapy Using Genetically Modified CD8+ HIV-Specific T Cells for HIV-Seropositive Patients Undergoing Allogeneic Bone Marrow Transplant.*
Date of RAC Approval: 2-11-92
Date of NIH Approval: 4-17-92

9206-018 (T) Brenner, Malcolm K.; Furman, Wayne; Santana, Victor; Bowman, Laura; and Meyer, William, St. Jude Children's Research Hospital; *Phase I Study of Cytokine-Gene Modified Autologous Neuroblastoma Cells for Treatment of Relapsed/Refractory Neuroblastoma.*
Date of RAC Approval: 6-1-92
Date of NIH Approval: 8-14-92

9206-019 (T) Oldfield, Edward, National Institutes of Health; *Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir.*
Date of RAC Approval: 6-1-92
Date of NIH Approval: 8-14-92

9206-020* (M) Deisseroth, Albert B., MD Anderson Cancer Center; *Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy.*
Date of RAC Approval: 6-2-92
Date of NIH Approval: 8-14-92
Minor Modification: 4-19-93

9206-021 (T) Gansbacher, Bernd; Houghton, Alan; and Livingston, Philip, Memorial Sloan Kettering Cancer Center; *Immunization with HLA-A2 matched Allogeneic Melanoma Cells that Secrete Interleukin-2 in Patients with Metastatic Melanoma.*
Date of RAC Approval: 6-2-92
Date of NIH Approval: 8-14-92

9206-022* (T) Gansbacher, Bernd; Motzer, Robert; Houghton, Alan; and Bander, Neil, Memorial Sloan Kettering Cancer Center; *Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma.*
Date of RAC Approval: 6-2-92
Date of NIH Approval: 8-14-92
Minor Modification: 3-31-93

9206-023 (M) Dunbar, Cynthia, National Institutes of Health; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Multiple Myeloma.*
Date of RAC Approval: 6-2-92
Date of NIH Approval: 8-14-92

9206-024 (M) Dunbar, Cynthia, National Institutes of Health; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Metastatic Breast Cancer.*
Date of RAC Approval: 6-2-92
Date of NIH Approval: 8-14-92

9206-025 (M) Dunbar, Cynthia, National Institutes of Health; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia.*
Date of RAC Approval: 6-2-92
Date of NIH Approval: 8-14-92

9209-026 (M) Walker, Robert E., National Institutes of Health; *A Study of the Safety and Survival of the Adoptive Transfer of Genetically Marked Syngeneic Lymphocytes in HIV Infected Identical Twins.*
Date of RAC Approval: 9-14-92
Date of NIH Approval: 9-3-93

9209-027 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center; *Phase I/II Study of the Use of Recombinant Human Interleukin 3 (rhil3) Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Breast Carcinoma or Hodgkin's Disease.*
Date of RAC Approval: 9-14-92
Date of NIH Approval: 2-5-93

9209-028 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center; *Evaluation of the Use of Recombinant Human G-CSF Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Lymphoid Malignancies.*
Date of RAC Approval: 9-14-92
Date of NIH Approval: 2-5-93

9209-029 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center; *A Trial of G-CSF Stimulated Peripheral Blood Stem Cells for Engraftment in Identical Twins.*
Date of RAC Approval: 9-14-92
Date of NIH Approval: 2-5-93

9209-030 (M) Deisseroth, Albert B., University of Texas MD Anderson Cancer Center; *Use of Retroviral Markers to Evaluate the Efficacy of Purging and to Discriminate Between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Neoplastic Cells in Autologous Marrow Following Purging in Patients with Chronic Lymphocytic Leukemia (CLL).*
Date of RAC Approval: 9-14-92

9209-031 (T) Roth, Jack A., The University of Texas MD Anderson Cancer Center; *Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer.*
Date of RAC Approval: 9-15-92

9209-032 (M) Brenner, Malcolm K., St. Jude Children's Research Hospital; A *Phase II Trial of the Baxter Neuroblastoma Bone Marrow Purging System Using Gene Marking to Assess Efficacy.*
Date of RAC Approval: 9-15-92
Date of NIH Approval: 2-5-93

9209-033 (T) Lotze, Michael T. and Rubin, Joshua T., University of Pittsburgh; *Gene Therapy of Cancer: A Pilot Study of IL-4 Gene Modified Antitumor Vaccines.*
Date of RAC Approval: 9-15-92
Date of NIH Approval: 2-5-93

9212-034* (T) Crystal, Ronald G., National Heart, Lung, and Blood Institute; *A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.*
Date of RAC Approval: 12-3-92
Date of NIH Approval: 4-16-93
Minor Modification: 5-17-93

9212-035* (T) Wilson, James M., University of Michigan; *Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial.*
Date of RAC Approval: 12-3-92
Date of NIH Approval: 8-26-93
Minor Modification: 8-17-93

9212-036 (M) Welsh, Michael J., Howard Hughes Medical Institute and Smith, Alan E.; Genzyme Corporation; *Cystic Fibrosis Gene Therapy Using an Adenovirus Vector: In Vivo Safety and Efficacy in Nasal Epithelium.*
Date of RAC Approval: 12-4-92
Date of NIH Approval: 4-16-93

9303-037 (T) Culver, Kenneth W., Iowa Methodist Medical Center, Des Moines, Iowa, and Van Gilder, John C.; University of Iowa, Iowa City, Iowa; *Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System.*
Date of RAC Approval: 3-1-93
Date of NIH Approval: 4-16-93

9303-038 (M) Heslop, Helen E.; Brenner, Malcolm K.; and Rooney, Cliona, St. Jude Children's Research Hospital, Memphis, Tennessee; *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T Lymphocytes to Recipients of Mismatched-Related or Phenotypically Similar Unrelated Donor Marrow Grafts.*
Date of RAC Approval: 3-2-93
Date of NIH Approval: 4-16-93

9303-039 (M) Brenner, Malcolm K.; Krance, Robert; Heslop, Helen E.; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *Assessment of the Efficacy of Purging by Using Gene-Marked Autologous Marrow Transplantation for Children with Acute Myelogenous Leukemia in First Complete Remission.*
Date of RAC Approval: 3-2-93
Date of NIH Approval: 4-16-93

9303-040 (T) Simons, Jonathan, Johns Hopkins Oncology Center, Baltimore, Maryland; *Phase I Study of Non-Replicating Autologous Tumor Cell Injections Using Cells Prepared With or Without Granulocyte-Macrophage Colony Stimulating Factor Gene Transduction in Patients with Metastatic Renal Cell Carcinoma.*
Date of RAC Approval: 3-1-93

9303-041 (T) Wilmott, Robert W. and Whitsett, Jeffrey, Children's Hospital Medical Center, Cincinnati, Ohio, and Trapnell, Bruce; Genetic Therapy, Inc., Gaithersburg, Maryland; *A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Human Cystic Fibrosis Transmembrane Conductance Regulator cDNA to the Airways.*
Date of RAC Approval: 3-2-93
Date of NIH Approval: 4-16-93

9303-042 (T) Boucher, Richard C. and Knowles, Michael R., University of North Carolina, Chapel Hill, North Carolina; *Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus: A Phase I Trial in the Nasal Cavity.*
Date of RAC Approval: 3-2-93

9306-043 (T) Seigler, Hilliard F., Duke University Medical Center, Durham, North Carolina; *A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients With Disseminated Malignant Melanoma.*
Date of RAC Approval: 6-7-93
Date of NIH Approval: 9-3-93

9306-044 (T) Deisseroth, Albert B.; Kavanagh, John; and Champlin, Richard, University of Texas MD Anderson Cancer Center, Houston, Texas; *Use of Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial.*
Date of RAC Approval: 6-7-93

9306-045 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *Immunotherapy for Cancer by Direct Gene Transfer into Tumors.*
Date of RAC Approval: 6-7-93
Date of NIH Approval: 9-3-93

9306-046 (T) Barranger, John A., University of Pittsburgh, Pittsburgh, Pennsylvania, *Gene Therapy for Gaucher Disease: Ex Vivo Gene Transfer and Autologous Transplantation of CD34(+) Cells.*
Date of RAC Approval: 6-7-93
Date of NIH APproval: 9-3-93

9306-047 (T) Karlsson, Stefan and Dunbar, Cynthia, NIH, Bethesda, Maryland, and Kohn, Donald B., Childrens Hospital Los Angeles, Los Angeles, California; *Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease.*
Date of RAC Approval: 6-7-93
Date of NIH Approval: 9-3-93

9306-048 (T) Galpin, Jeffrey E., University of Southern California, and Casciato, Dennis A., University of California, Los Angeles, California; *A Preliminary Study to Evaluate the Safety and Biologic Effects of Murine Retroviral Vector Encoding HIV-1 Genes [HIV-IT(V)] in Asymptomatic Subjects Infected with HIV-1.*
Date of RAC Approval: 6-7-93
Date of NIH Approval: 9-3-93

9306-049 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *A Molecular Genetic Intervention for AIDS - Effects of a Transdominant Negative Form of Rev.*
Date of RAC Approval: 6-7-93
Date of NIH Approval: 9-3-93

9306-050 (T) Raffel, Corey, Childrens Hospital Los Angeles, Los Angeles, California, and Culver, Kenneth, Iowa Methodist Medical Center, Des Moines, Iowa; *Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene.*
Date of RAC Approval: 6-8-93
Date of NIH Approval: 9-3-93

9306-051 (T) Hesdorffer, Charles and Antman, Karen, Columbia University College of Physicians and Surgeons, New York, New York; *Human MDR Gene Transfer in Patients with Advanced Cancer.*
Date of RAC Approval: 6-8-93
Date of NIH Approval: 9-3-93

9306-052 (T) Ilan, Joseph, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio; *Gene Therapy for Human Brain Tumors Using Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I.*
Date of RAC Approval: 6-8-93

M = Human Gene Marking Protocol
T = Human Gene Therapy Protocol
* = Minor Modification
** = Major Amendment
_ = RAC Approved/NIH Approval Pending

	T	M	Total (T + M)
RAC APPROVED	29	23	52
NIH DIRECTOR APPROVED	24	22	46

September 7, 1993

MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS

DATE OF MINOR MODIFICATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
3/30/90	3/2/89	8810-001	Rosenberg, Steven A.	The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2, and Tumor Infiltrating Lymphocytes	Lift the cap on the number of patients accrued onto the protocol.
11/91	7/12/91	9102-004	Brenner, Malcolm K., et. al.	Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-005	Brenner, Malcolm K., et. al.	A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-006	Brenner, Malcolm K., et. al.	A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement	Use the retrovirus vector, G1Na, instead of LNL6.
7/9/92	10/15/91	9110-010	Rosenberg, Steven A.	Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF)	Extend patient eligibility criteria to include patients with advanced breast cancer.
11/30/92	1/17/92	9105-009	Lotze, Michael T.	The Administration of Interleukin-2~~, Interleukin-4,~~ and Tumor Infiltrating Lymphocytes to Patients with Melanoma	Eliminate systemic IL-4 therapy.
12/3/92	11/14/91	9110-012	Wilson, James M.	Ex Vivo Gene Therapy of Familial Hypercholesterolemia	Increase patient accrual from 3 to 5 patients.
1/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	(1) addition of assays for anti-DNA antibodies on day 28 and week 8; (2) repeat chest and abdominal CTs, when indicated, at 6-8 weeks following initial treatment; (3) additional biopsies perfomed on days 16 and 20 and as needed to verify gene expression; (4) reduce the duration of the protocol from 16 to 8 weeks; and (5) expand patient eligibility to include those patients who have tolerated previous treatments without side effects at higher doses.
1/22/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	Deliver HLA-B7 DNA liposome complexes by catheter to pulmonary nodules.

DATE OF MINOR MODIFI-CATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
3/31/93	8/14/92	9206-022	Gansbacher, Bernd, et. al.	Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma	Use only irradiated SK-RC-39 cell line, not SK-RC-28.
4/19/93	7/12/91	9102-007	Deisseroth, Albert B.	Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial	New transduction protocol which allows the use of autologous stroma and growth factors.
4/19/93	8/14/92	9206-020	Deisseroth, Albert B.	Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy	New transduction protocol which includes the use of autologous stroma and growth factors.
4/21/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells	Treat newborns with ADA deficiency with ADA gene-corrected autologous hematopoietic stem cells obtained from the patients' umbilical cord and placenta.
5/17/93	4/16/93	9212-034	Crystal, Ronald G.	A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient. Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.	Decrease the "baseline" and "vehicle control" portions of the protocol from 1 month to 1 week each.
6/7/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells	The investigators are permitted to: (1) withdraw PEG-ADA at their discretion, and (2) perform bone marrow aspirations as necessary to monitor for gene transduction.
8/6/93	2/5/93	9202-016	Freeman, Scott M.	Gene Transfer for the Treatment of Cancer	PI has moved to Tulane University Medical Center. The total number of patients will be divided between Tulane University and the University of Rochester.
8/17/93	8/25/93	9212-035	Wilson, James M.	Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.

DATE OF MINOR MODIFI-CATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
8/19/93	11/14/91	9110-012	Wilson, James M.	Ex Vivo Gene Therapy for Familial Hypercholesterolemia	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.

ORDA/August 27, 1993

9309-053 Cassileth, Peter; Podack, Eckhard R.; Sridhar, Kasi; University of Miami, and Savaraj, Niramol; Miami Veterans Administration Hospital, Miami, Florida; *Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene Product in Limited Stage Small Cell Lung Cancer.*

Date of RAC Approval: 9/9/1993
Date of NIH Approval: 12/2/1993

SCIENTIFIC ABSTRACT

Small-cell lung cancer is a fatal disease in more than 90% of affected patients despite combined modality therapy with radiation and chemotherapy. The intent of this protocol is to culture small-cell lung cancer cells and transfect them with the gene for Interleukin-2 (IL-2) production. These transfected cells will be returned to patients after cytoreduction of their cancer with conventional chemotherapy. The aim is for the reimplanted, IL-2 producing tumor cells to stimulate the proliferation of a tumor-specific population of cytotoxic lymphocytes capable of destroying the reimplanted tumor. The plasmid vector BMG-Neo is an efficient vector of transfection. It uses as a eukaryotic replication unit an 85.5 kb DNA fragment from the bovine papilloma virus 1. This plasmid replicates extra chromosomally as an episome producing a copy number of 20-100 per cell. In pre-clinical studies in a murine model using the Lewis lung carcinoma cell line, the administration of IL-2 transfected cancer cells led to rejection of subsequently administered non-transfected tumor cells and to regression of established tumors. In this study in humans, IL-2 transfected radiated (to prevent proliferation) tumor cells will be serially injected weekly over 4-6 weeks. Companion laboratory studies will seek to quantify and characterize the nature of the induced cytotoxic lymphocyte response in the patients' peripheral blood. The laboratory component, in part, also will clone out populations of tumor specific lymphocytes for in vitro transfection studies of these cells. Clinical measures of response include regression of established tumor deposits and/or delay in time to disease progression.

NON-TECHNICAL ABSTRACT

Small-cell lung cancer is a rapidly growing variant of lung cancer that is fatal in more than 90% of patients within two years of diagnosis despite treatment with both chemotherapy and radiation therapy. There is laboratory and clinical evidence in animal systems indicating that under the right circumstances immune cells, called lymphocytes, can be stimulated to proliferate, recognize, and destroy cancer cells. In clinical studies in humans, injection of Interleukin-2 in high doses stimulates lymphocyte growth and causes shrinkage of highly resistant cancers in some patients, albeit with substantial side effects. In this study, small-cell lung cancer cells from patients will be grown in the test tube and the gene for interleukin-2 will be placed into these cells by means of a carrier to transport the gene, called a plasmid. The tumor cells, now carrying the gene for production of interleukin-2 are radiated so that they are alive but cannot grow and then are returned by injection under the skin into the patient. The intent is to have the tumor cell produce interleukin-2 locally and thereby stimulate sets of lymphocytes that are specifically geared to recognize the tumor cells and destroy them. By means of repeated injections of these gene-altered cells, it is hoped that adequate numbers of stimulated lymphocytes will circulate and cause the destruction of the remaining unaltered lung cancer cells in the patient's body. Toxicity is expected to be minimal because of the low doses of Interleukin-2 generated by the cells. Patients will be carefully monitored for any adverse effects and to determine if clinical response occurs.

Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene Product in Limited Stage Small Cell Lung Cancer

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Research Nurse: James Hanlon, R.N.

Estimated Duration of Study: 2 years

Number & Kind of Subjects Needed:

Number	Sex	Age
12	M&F	18 & older

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July 14, 1993

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1. BACKGROUND AND PRELIMINARY STUDIES

1.1 Introduction

Immunologic surveillance (1) is believed to be one means by which cells undergoing malignant transformation are eliminated from the body. Although established cancers escape this potential control mechanism, the possibility that autologous cellular immunity could be stimulated to kill cancer cells led to a number of therapeutic trials of interleukin (Il) - 2 (1). These studies demonstrated that responses could be obtained even in highly resistant neoplasms, such as advanced renal cancer and melanoma. Although the response rate was low, and the responses generally partial and short-lived, some lengthy complete remissions resulted. The effectiveness of Il-2 was not enhanced by the co-administration of ex vivo cultivated LAK (lymphokine activated killer) cells or TIL (tumor infiltrating lymphocytes) (2). Investigators are currently exploring the value of markedly reduced doses of Il-2 to diminish the substantial toxicity that results from its systemic administration. Developments in gene transfection present an alternate approach to modulate the immune response to cancer. It is now feasible to insert the genes for the production of interleukins directly into cells. After transfection, these cells can be returned to the patient where interleukins can be produced locally to induce an anti-tumor response, as has been demonstrated in animal systems at the University of Miami (3) and elsewhere (4,5). The intent is that lymphocytes activated locally by a combination of interleukins and tumor surface antigens will yield a tumoricidal response not only to the administered transfected interleukin-expressing cancer cells but also to the nontransfected cancer cells remaining in the patient.

1.2 Background

The benefit from conventional cancer treatment modalities has plateaued, and new directions are needed to improve the outcome for patients with cancer. Immunologic modulation of the host in order to develop an autologous cytocidal attack on the cancer cells is an increasingly appealing alternative to the modalities of chemotherapy and radiation therapy.

White blood cells that are capable of lysing cancer cells include natural killer (NK) cells, lymphokine-activated killer (LAK) cells, cytotoxic T lymphocytes (CTL), and activated macrophages. Of these, the cancerocidal activities of LAK cells have been the most extensively studied, largely through the work of Rosenberg and colleagues (1). The potent lymphokine, interleukin-2 (Il-2), produces LAK cells from precursor lymphocytes and stimulates LAK cell proliferation. The LAK cells have been characterized phenotypically as non-adherent, non-NK (5) cells that were non-T, non-B and HLA class II negative (6). It now appears that Il-2 exposure causes either the generation of CD3- (T cell marker), CD16+ (Fc receptor for polymeric IgG) LAK cells by activation of NK cells (CD3-,CD16+) (7-11) or CD3+,CD16- LAK cells from activated T cells (7,12). The proportion of the different types of LAK cells and whether increased NK cell production also occurs after Il-2 stimulation depends on the setting (in vivo or in vitro) and dose and duration of exposure to Il-2 (7,13,14). Functionally, in vitro, LAK cells lyse NK-sensitive as well as NK-resistant cell lines (15) and cells that do not express detectable tumor specific antigens (14). Despite their broad range of lytic capability, LAK cells are not non-specific killer cells. In vitro, they lyse autologous tumor cells and fresh allogeneic tumor cells, but not normal lymphocytes (15). LAK cells are lytic for both autologous and allogeneic acute myeloid and lymphoid leukemia and lymphoma cells (16). Although exposure to antibodies against HLA Class I and Il antigenic determinants blocks this activity against allogeneic targets, autologous cell target kill is unaffected. This lack of HLA-restricted cell killing makes it possible for LAK cells to destroy tumor cells that fail to or weakly express histocompatibility antigens (17). At the same time, it is well to realize that the subset of LAK cells derived from T lymphocytes does require HLA class I antigen expression for maximum effectiveness.

Rosenberg concluded that adoptive immune therapy by means of Il-2 stimulated LAK cells could overcome defective cellular immunity to cancer (6,18). In a variety of murine systems, he showed that the administration of Il-2 plus ex vivo amplified LAK cells led to marked regression of disseminated cancers (6,19,20) and cure of leukemia with (21) or without syngeneic marrow transplant (22). His studies also revealed the pleiotropic effects of Il-2, which include: endothelial cell antigen activation; stimulation of B cell proliferation; increased HLA class II (Dr) antigen expression on endothelial cells, tumor infiltrating lymphocytes (TIL), and melanoma cells; eosinophilia; and enhanced production and release of tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) (9). Some of these may play important enhancing roles in the immune response to Il-2. Il-2 administration (with or without ex vivo generated LAK cells) in humans caused overall responses (including some durable complete remissions) in the range of 15-30% in patients with far-advanced, highly resistant neoplasms, such as melanoma and renal cancer (23-26) and relapsed acute leukemia (27). The high doses of Il-2 used in the initial studies (even without reinfusion of cultured LAK cells) were associated with substantial toxicity, including capillary leak syndrome, severe hypotension, renal failure, liver injury and pulmonary insufficiency requiring intubation (26,28-31). This led to trials employing lower doses and/or continuous infusions (13,14,32-35) of Il-2. Toxicity, although reduced, still persisted, limiting the duration of therapy, and possibly the response rates as well. Attempts to achieve greater specificity and response by utilizing Il-2 plus TILs, harvested from the lymphocytes infiltrating some cancers, have thus far been technically difficult and associated with toxicity (2,9). The short half-life of Il-2, the toxicity of its systemic administration, and the rapid disappearance of effector cells after termination of Il-2 are major limiting features.

The finding of tumor infiltrating lymphocytes in many tumors, or even in circulation, which however do not mount a effector response in vivo, could suggest that these lymphocytes may be specifically recognizing tumor cells but remain anergic or suppressed (36-38). According to current models, anergy in T-cells may be induced by the binding of ligands to the T-cell receptor variable region in the absence of second signals, which usually are provided by lymphokines (For review, 39).

In support of such a hypothesis are the studies by Fearon et al. (40), Tepper et al. (41), Gansbacher et al. (42) demonstrating that nonimmunogenic tumors, upon transfection with IL2 or IL4, respectively, are rejected by syngeneic mice, whereas the normal (untransfected) tumor invariably kills the mice in a short period. These findings suggest that the production of lymphokines by the tumor can lead to its rejection. In other words a nonimmunogenic tumor seems to be rendered immunogenic upon lymphokine production by the tumor cells. Interestingly, tumor cell IL4 production caused tumor rejection even in nude mice suggesting the stimulation of non specific, inflammatory processes, involving mostly eosinophiles and monocytes.

1.3 University of Miami Pre-Clinical Studies

a. Effect of Il2, Il4, Il6 and TNF transfection on the rejection of Lewis lung carcinoma

We have completed a number of studies to determine whether the nonimmunogenic Lewis lung carcinoma (LLC) could be rendered immunogenic by transfection with the lymphokine cDNAs for Il2, Il4, Il6 and TNF and subsequent reinjection into murine syngeneic recipients (3,4 manuscript in appendix). LLC is a spontaneous lung tumor in C57Bl6 (43) which, when injected subcutaneously (10^6 cells), will kill all recipients with a mean survival time of 34 days. No immune response is apparent and no infiltrating cells are detectable.

Unlike in studies by others, we used for gene transfer a plasmid vector system (pBMG-Neo) rather than a retroviral vector. The plasmid vector system pBMGNeo (44) is advantageous because it appears safer and, owing to its copy number of 20 -100 per cell, it allows higher levels of expression than single copy vectors. The vector system is described in detail below. Briefly, the cDNA is expressed under the metallothionein promotor, which is active in a wide variety of cells. The plasmid replicates as episome under the influence of the 69% fragment of the bovine papilloma virus type 1 (BPV1). The L1 and L2 late gene products of BPV1, coding for structural viral proteins, have been deleted, disabling the potential of the plasmid system to produce intact, infectious virus particles. An additional safety factor is the stringent species specificity of papilloma viruses.

Transfection of murine LLC with pBMG-Neo-cDNA was achieved by the Lipofectin (BRL) technique. Stable and high levels of expression for all cytokines was observed using as inserted cDNAs murine Il2, murine Il4, and human Il6 (Table 1, see Appendix C). These studies confirm the original description of pBMG-Neo as an excellent, eukaryotic expression vector, (Table 1, see Appendix C). The doubling time of transfected cells is not changed which demonstrates that the vector has no significant and consistent effect on the proliferation of LLC.

TNF, contained in the vector pCDV-TNF (SV40 based) which integrates into the genome and expresses TNF under the SV40 promotor, showed variable levels of expression from one transfection to the next (Table 2, see Appendix C). In addition the levels of expression with this vector are low and sometimes undetectable. Table 2 (see Appendix C) also shows that in double transfection experiments, using pBMG-Neo-Il2 and pCDV-TNF, Il2 expression is consistently high whereas TNF is quite variable. It is evident that the transfected and selected cells are able to secrete cytokines. Therefore a low level of TNF production is likely due to the low copy number of the vector and/or to the locus of insertion in the genome. For the purpose of gene therapy non integrating vectors with an intermediate copy number may be superior in the expression of the gene product, when compared to single copy Indeed, episomal plasmids usually produce 50 to 100-fold higher levels of the gene product than single copy vectors(45). In addition episomal vectors do not carry the risk of insertional mutagenesis.

b. Rejection of LLC-Il2

Subcutaneous injection of C57Bl6 mice with 10^6 normal LLC or vector transfected LLC-Neo leads to tumor growth and death with a median survival of 33.3 days. Simultaneous, systemic application of recombinant Il2 in various schedules and doses does not prolong survival. Subcutaneous injection of Il2 *transfected* LLC in contrast results in rejection of the Il2 producing tumor cells and survival of the mice (Table 3, see Appendix C) up to a tumor cell dose of 10^7/mouse.

Only normal, immunocompetent mice were able to reject the Il2 producing LLC cells. LLC-Il2 was not rejected in syngeneic NK deficient mice (Beige) nor in T-cell deficient mice (Nude) nor in SCID mice (See manuscript in appendix). That the immunodeficient animals attempted to mount an immune response can be deduced from the fact that they survived longer upon injection of Il2 producing LLC (70 days) when compared to the injection of the parent tumor (Table 4, see Appendix C). It is important to note that the vector or vector plus insert transfected cells do not decrease the survival time of injected mice. This indicates that the viral vector does not seem to increase the in vivo malignancy of the cells. This is in accord with a lack of influence on the doubling time in vitro and measurable growth rate in vivo.

These data indicate that for the rejection of LLC-Il2 both an effective T-cell response and an NK cell response is required; a B-cell response is not essential since SCID mice, lacking both T and B cells, have the same survival times as Nude mice, lacking only T cells.

c. LLC-Il2 delays growth of untransfected LLC

Several studies have already established that the rejection of Il2 producing tumors establishes a protective immunity to a subsequent tumor challenge with normal tumors. We wished to determine whether the immunization with LLC-Il2 was able to reject or delay the growth of already established LLC at a different site. This question approaches the situation in human tumors, attempting to influence tumor growth of already established tumors by immunization. Injection of $5x10^6$ LLC-Il2 on days 1, 3, 5, 7 after transplantation of LLC into the contralateral side resulted in prolongation of survival from 33.6 ± 6.7 to 47.8 ± 4.7 days ($p<0.01$) see manuscript, Appendix E. This is a significant therapeutic effect, that we hope to improve by further modification of the injection dose, schedule and combination of lymphokines. It will be possible for instance to increase the dose of injected LLC-Il2 by first irradiating the cells so as to prevent their outgrowth as tumor.

d. Generation of a CD4 cytotoxic response by LLC-Il2

Immunization of mice with $5x10^6$ LLC-Il2 on day 1, 3, 5, 7 and 49, followed one week later by the analysis of tumor specific cytotoxicity in unfractionated spleen cells, generated 20% tumor lysis at a 100:1 spleen cell to tumor cell ratio in 4h Cr-release assays. Both LLC and LLC-Il2 could serve as targets. Spleen cells from control animals had no cytotoxic activity towards either LLC or LLC-Il2. The cytolytic activity of spleen cells from immunized mice was completely blocked by anti CD4 antibodies but not by anti CD8 antibodies added during the assay (Fig.1, see Appendix C). In accord with this finding we observed a expression of MHC class II antigen on LLC, which did not change upon transfection with pBMG-Neo-Il2 (Fig.2, see Appendix C). The summary of cytokine transfection on the rejection rate of LLC, see Table 5, Appendix C).

As far as combinations are concerned the combined use of Il2 and Il4,based on our model studies, appears promising. In addition it is believed that Il2 and Il4 are necessary for the generation of a memory CTL response and it has been reported that the presence of Il4 together with Il2 suppresses a promiscuous CTL response and generates instead an antigen restricted response. This hypothesis will be further tested in the laboratory.

We have not yet conducted studies with γ-Ifn in our LLC model. However this lymphokine appears attractive in combination with Il2, since it is known to upregulate MHC expression, required for T-cell recognition, and to increase cytotoxic activity. We are therefore in the process of including γ-Ifn in our studies.

1.4 Human Small Cell Lung Cancer

Lung cancer is one of the most common (150,000 new cases per year) and most lethal (130,000 deaths per year) cancers in the U.S.A. (46). The two major classes of lung cancer are non-small cell carcinoma (NSCLC) and small cell carcinoma (SCLC); the latter accounts for 1/4 of all lung cancers. Combination chemotherapy $\pm$ radiation therapy is the treatment of choice for SCLC, which is a rapidly progressive disease that disseminates widely and early. Unlike complex staging classifications of most other cancers, the stages of SCLC consist only of limited disease (LD) and extensive disease (ED). LD refers to SCLC which is confined largely to one hemithorax so that the identifiable areas of disease can be encompassed in a single radiation therapy treatment field. ED includes any disease outside the

boundaries of LD disease. LD stage SCLC accounts for approximately 1/3 of all SCLC. Although initial chemotherapy in ED produces a 65-85% overall response and a 15-30% complete response, median survival is short (7-11 months) and two year survival is < 5% (46-50). Because of the rapid pace and brief duration of response to therapy, ED patients will not be well enough, long enough to allow generation and therapy with gene transfected cells. We will therefore include only patients with LD on this study. Initial chemotherapy produces an overall response of 80-95% in patients with LD and a complete remission rate of 40-70% (46,51-54). The median survival is 12-16 months, two year survival, 10-25%, and 5 year survival, <10% (47). A range of chemotherapy regimens have been employed in therapy, most commonly cyclophosphamide, adriamycin and vincristine (CAV) or etoposide and cis-platinum (EP) (46). Neither regimen used alone or together is superior (50).

Although the addition to chemotherapy of radiation therapy to the primary site of disease in the lung increases the complete remission rate, median and long-term survival is probably not improved (46). Moreover, in one study, delayed radiation therapy was better than early, concurrent radiation therapy (56). The results of studies using cyclophosphamide, adriamycin and etoposide as we use here showed comparable results without primary radiation therapy (55). Similarly, although prophylactic cranial irradiation reduces the chances of later symptomatic brain metastases, survival is not improved (46). Because long-term outcome is unaltered and to avoid suppression of the immunologic response, radiation therapy will not be used in the initial therapy of these patients, prior to gene therapy.

1.5 Establishment of primary lung tumor cell cultures from patients

The development of techniques to grow SCLC cell lines has been greatly improved. At present over 50 SCLC cell lines have been established and studied (56-61) The success rate in growing SCLC is approximately 70-80%. Based on the appearance of the cell culture, SCLC cell lines can be classified into two major groups (62,63). The so-called "classical small cell lung cancer" will grow as spherical or dense aggregate (~80%). These cell lines express properties of APUD cells and contain high levels of the neurone specific enolase and L-dopadecarboxylase. Generally, the "classical" SCLC cell lines have been established from specimens of patients who had the usual course of disease with response to chemotherapy. The other "variant" of SCLC is usually associated with a more malignant course and poor prognosis. These cells tend to grow loosely aggregated or attach as monolayer and do not secrete peptide hormones. They also tend to have amplification of certain oncogenes, such as C-myc or N-myc. In vitro, drug sensitivity testing using SCLC lines established from patients has demonstrated a good correlation with the patients' clinical response and survival (64-66). These observations suggest the exploration of innovative treatments in this disease. Established cell lines retain the genetic characteristics of SCLC tumor samples, such as deletion and/or LOH on chromosome 3p, abnormalities in structure and expression of the Rb gene (13% in primary tumor and 18% in SCLC lines) and mutation of p53 (67-69).

The ability of SCLC to grow in vitro has been shown not to correlate with the prognosis of the patient (70); in contrast the ability to establish non SCLC (NSCLC) lines correlates with decreased survival (71). Therefore, there will be no bias in SCLC for patients with poor prognosis. All these considerations have led us to choose SCLC as a model for this trial. Although the median survival of SCLC is relatively short (8 months), patients usually respond to chemotherapy. We estimate from our experience that a 4 month period is required to establish cells and to transfect and select them, which should suffice for those patients who are responsive to chemotherapy.

During recent years, we have cultured SCLC from lymph nodes, pleural effusion, peritoneal effusion, and bone marrow from 36 patients at the Miami V.A. Medical Center. From six patients we obtained samples twice (at the time of diagnosis and relapse), from one patient we obtained two samples, from bone barrow and from pleural fluid, and from one patient we obtained three samples, two from bone marrow at diagnosis and at relapse, and one from peritoneal fluid. We were able to establish 6 permanent cell lines and 2 short term cultures (6 months) from 11 samples with histologically or cytologically confirmed diagnosis of SCLC in the bone marrow or malignant effusion. Three bone marrow samples with histological confirmed diagnosis of SCLC did not grow; one of these samples from a previously treated patient. On the other hand, we were able to establish cultures from three patients with extensive SCLC whose bone marrow biopsies appeared normal. Similar results also have been published by Hunter et al. (72). He reported 22% positive tumor colony growth in histologically negative marrows. Furthermore, studies using one to three different monoclonal antibodies have documented a 28%-43% increased sensitivity in detecting bone marrow metastasis over conventional light microscopy technique.

A summary of our culture results (73-75) is shown in Table 6 (see Appendix C). The characteristics of our SCLC lines are summarized in Table 7 (see Appendix C).

All cell lines show abnormalities in 3p chromosome and homozygosity loss at the DNF15S1 (3p21) loci by Southern analysis, in association with other chromosomal structural abnormalities. The chemosensitivity of these cell lines has been described (76,77).

1.6 MHC expression of and lymphokine production by transfected SCLC

To date we have analyzed four recently established SCLC lines, one of which grows as adherent cell type and the other three as loose aggregates. Fig. 3, 4 (see Appendix C) shows the FACS analysis for MHC class I and class II expression in comparison to nonspecific IgG binding. Constant region recognizing MHC antibodies were used. All four SCLC lines express class I. Class II MHC is also present except in line one, where it is barely detectable above background. It is of interest to note the resemblance to the murine LLC also with regard to the apparent membrane shedding and the ability to grow semi adherent.

Transfection and selection in G418 does not significantly effect the cell surface expression of MHC molecules Fig. 4 (Appendix C). IL2 production in SCLC#1 was 5720U/ml $5x10^6$ cells x 24hr.

SCLC-hIl2#1 was irradiated at 0, 1500, 3000, 6000, and 12000 rad and plated in microtiter plates at $5X10^4$cells/well. For the next ten days parallel samples were counted tested for Il2 (Table 9a, b; see Appendix C). We found that by day 10 Il2 production had dropped to zero in parallel with loss of viability in cells irradiated with 6000 and 12000rad (Table 9b, Appendix C). For the first four days little decline in Il2 production was observed (350U/well). By day 8 the production was still 90U/well. These studies indicate that proliferation is not necessary for Il2 production and hence that lethally irradiated, transfected tumor cells ought to function well in the induction of an immune response.

These data indicate that SCLC a. is transfectable similar to LLC; b. that transfection and selection does not change the surface expression of MHC; c. that SCLC produces high levels of lymphokines; and d. that 12000rad irradiation results in slow cell death, allowing the production of lymphokine for at least eight days. SCLC cell lines thus fulfill the necessary criteria for gene therapy.

2.0 PROPOSAL PROTOCOLS AND EXPERIMENTAL DETAILS

In patients with limited stage small cell lung cancer, to:

2.1 Determine the toxicity and safety of administering the patients' own gene-transfected cancer cells that express Il-2.
2.2 Demonstrate the generation of local and systemic tumor-specific T effector lymphocytes capable of lysing the gene-transfected and nontransfected cancer cells.
2.3 Assess the patients for evidence of a clinical response to therapy.
2.4 Develop the framework for subsequent studies of gene-transfection directed at enhancing the intensity, durability and specificity of the autologous immunologic response to cancer.

3.0 ELIGIBILITY CRITERIA FOR ENTRY ON STUDY

Because it is essential that we select patients with an opportunity to remain in response long enough for unimpeded therapy with the gene transfected cells, we will exclude patients from study with poor prognostic features. In LD SCLC, established adverse prognostic variables include poor performance status, extensive weight loss, age $\geq$ 70 years, elevated LDH (serum lactic dehydrogenase level), and the presence of a pleural effusion (78). The eligibility criteria listed below reflect these considerations, and are geared to select a favorable subset of LD SCLC who will still be eligible for gene therapy 4-6 months after initial biopsy for cell culture.

Patients:

3.1 Must have a histologically or cytologically verified diagnosis of SCLC of Limited Disease Stage as determined by chest X-ray, CT scans of the chest and abdomen, CT or MRI scans of the brain, bone scan, and bone marrow examination.
3.2 Who have a pleural effusion or an elevated LDH are excluded from study.
3.3 Must have accessible tumor by lymph node biopsy or mediastinoscopy.
3.4 Must be $\geq$ 18 years old.
3.5 With a history of myocardial infarction within the preceding 6 months or with angina are excluded. Patients with a past history of cardiac disease, must have a normal cardiac ejection fraction by MUGA scan.
3.6 Must have a white blood cell count of $\geq$ 3,000/mm^3 and platelet count $\geq$ 100,000/mm^3.
3.7 Must have serum creatinine clearance $\geq$ 70 ml/min by determination or calculation from serum creatinine.
3.8 Must have a bilirubin $<$ 1.2 mg/dl and transaminases and alkaline phosphatase $<$ 2 times normal.
3.9 Must have an ECOG (Eastern Cooperative Oncology Group) performance status of 0 (no physical limitations) or 1 (only restricted from strenuous activity).
3.10 Who have lost $>$5% of their premorbid weight are excluded.
3.11 Who are women may not be pregnant or lactating. All patients with reproductive potential must agree to use an effective contraceptive method for the duration of the study.
3.12 With prior or current anti-tumor therapy are excluded.
3.13 With any prior malignancy, except for adequately treated basal or squamous cell skin cancer or in situ cervical cancer, are excluded.
3.14 With AIDS or HIV infection are excluded.

3.15 With active infection are excluded.
3.16 With active papilloma virus infection, including common warts or condyloma acuminata, will be excluded.
3.17 Must have signed informed consent.

4.0 TREATMENT PLAN

4.1 After complete staging and satisfaction of eligibility criteria, patients will undergo lymph node biopsy by surgery or by mediastinoscopy. Approximately 1/2 of the tissue will be sent for culture and establishment of a cell line, 1/4 will be sent to Pathology for histologic review, and 1/4 will be desegregated and cryopreserved for later use as a reagent for studies of immunologic response after therapy with gene transfected cells. In the event that the patient's tumor cells cannot be established in culture and stably transfected, the patient will go off study.

4.2 All patients will be treated with combination chemotherapy administered every three weeks, with dosage adjustments made as necessary based on pretherapy blood counts. Each course of chemotherapy will consist of the following:

Cyclophosphamide 1 gm/sq.M. IV	on Day 1
Doxorubicin 50 mg/sq.M. IV	on Day 1
Etoposide 120 mg/sq.M. IV	on Days 1,2, and 3

G-CSF (Filgrastim) 250 mcg/sq.M. subcutaneously daily starting on day 4 and continuing until day 17 (unless nadir neutrophil count is > 10,000/mm3 after Day 12, in which case G-CSF will be discontinued).

Patients will receive a minimum of four cycles of chemotherapy if they are responding and a maximum of two cycles beyond their best response. Patients with stable disease/no response or progressive disease will be taken off study. Responding patients in good partial remission or in complete remission will be eligible for return of their gene-transfected cells.

NOTE: These patients will receive approximately 3-4 months of chemotherapy. No further therapy will then be given, pending return of their gene transfected cells. We anticipate that the interval between the termination of chemotherapy and the opportunity to receive their gene-transfected cancer will be 2-3 months. In that interval, approximately 10-15% of patients in good partial remission or complete remission will relapse and need additional therapy. These patients will go off-study. The remainder should be in stable response to permit gene-therapy to occur at a time distant from the immunosuppressive effects of chemotherapy. Radiation therapy to the primary site of disease in the chest will be deferred until after completion of the gene therapy phase of this study, as discussed previously.

4.3 Establish new SCLC lines

Tumor cells will be obtained from bone marrow aspiration, bronchoscopy, mediastinoscopy, or thoracentesis. Must have accessible tumor by lymph node biopsy or mediastinoscopy.

Specimen Preparation:

Bone marrow aspirate will be collected into a syringe containing preservative-free heparin, diluted

with RPMI-1640 media. Mononuclear cells will be separated by Ficoll-Hypaque gradient centrifugation. Approximate $1x10^6$ cells will be suspended in culture media. The viability of the cells will be determined by trypan blue exclusion.

Solid tumor specimens: One portion of the tumor sample will be minced and passed through a tissue collector screen (80 gauge steel mesh, Belco Glass, Inc.) to form a single cell suspension and gently pipetting in RPMI-1640. The viability of cell preparation will be determined by trypan blue exclusion. The other portion of tissue will be cut into small pieces and placed on a six-well culture plate containing media which will be described in the next section. This latter method is to facilitate the faster growth of SCLC sample which may grow as monolayer. For tumor obtained via bronchoscopy, tissue will be washed with PBS supplemented with penicillin (500U/ml), streptomycin (500μg/ml), gentamycin (50μg/ml) and amphotericin-B (2 μg/ml) to avoid contamination.

4.3.1 Cell Culture:

Cells will be divided into two portions if adequate cell numbers are obtained. One portion will be placed in 6-well culture plates containing Aim V medium. The other portion will be supplemented hydrocortisone (10nM), insulin (5μg/ml), 17 -estradiol (10nM), and sodium selenite (30nM) as described by Carney et al (44,64,65).The plate will be incubated in a humidified CO_2 incubator. Cells will be examined twice weekly for viability and proliferation. Every 1-2 weeks half of the media will be replaced without aspirating the cells. If no growth can be visualized by microscopic examination, bombesin at 10 ng/ml will be added. When cells start to proliferate, 5% FBS will be added to the serum free, hormone and chemical supplemented media and finally switched to RPMI-1640 with 10% FBS when cell lines are established. At early passages, cells will be fed with 50% conditioned media and 50% fresh media. In our experience, SCLC cells begin to proliferate well and continuous passage can be established in about 3 months. These cells will be used for transfection. We realize that these cells may not be immortalized and that it may not be possible to establish permanent cell lines. However, SCLC can proliferate well up to 6-8 months and can thus be used for transfection. We will routinely examine these cells for mycoplasma contamination using the test described below. For cells to be reinjected to patients the services of the American Type Culture Collection (culture and staining) will be used (FDA required).

Testing for Mycoplasma contamination:

We will use the kit available from GIBCO-BRL (Gaithersburg,MD). The MycoTect Kit is based on the principal that mycoplasma contains significant amounts of adenosine phosphorylase, an enzyme which is almost absent in mammalian cells. Adenosine phosphorylase converts 6-methylpurine deoxyribose (6-MPDR), a nontoxic analog of adenosine, into 6-methylpurine and 6-methylpurine riboside, which are toxic to mammalian cells. A mycoplasma infected culture can be detected by incubation with 6-MPDR and monitoring for toxicity towards mammalian cells. This test does not substitute for later mycoplasma tests prior to patient treatment.

4.3.2 Immunohistochemical study:

SCLC is recognized as neuroendocrine tumor and usually express a multiple neuroendocrine markers such as neuron-specific enolase (NSE), Leu-7, chromogranin-A (79-81). NSE and chromogranin-A will be detected by immunohistochemical staining using the commercially available antibody and the sensitive avidin-biotinylated peroxidase technique. Cells growing as nonadherent aggregates will be collected on slides by the cytospin technique. Cells growing as monolayer will be seeded on cover-slips or plated in the slide- with-chamber from LabTek (Miles laboratories).

L-dopa decarboxylase (DDC) is another useful marker in SCLC tumors, especially in "classical SCLC" (82,83). The DDC assay will be performed using the method published previously (84). Briefly, the supernatant extract from the cell homogenate will be mixed with [carboxyl]^{14}C labeled dopa together with pyridoxal phosphate and 0.1M Tris buffer pH 9.0 in an Eppendorf tube placed inside a glass scintillation vial containing hyamine hydroxide for trapping ^{14}CO2. Perchloric acid (2N) is added to terminate the reaction and to convert H^{14}CO2 to free $^{14}CO_2$. Enzyme activity will be determined by measurement of CO_2 release from ^{14}C labelled L-dopa. One unit of enzyme activity is defined as 1 nmol of $^{14}CO_2$ /hr/mg of protein.

4.4 IL-2 cDNA CONSTRUCT AND EXPRESSION VECTOR:

4.4.1 IL2 cDNA constructs

Human Il2 cDNA is modified by PCR in the following way. The 5' untranslated sequence 1-89 is truncated. Position 90 is preceded by the Sal 1 recognition sequence for cloning. Position 90 to 572 correspond to the natural Il2 sequence followed by the Sal 1 recognition sequence. Compared to the natural Il2 mRNA the construct lacks 89 nucleotides of the 5' untranslated sequence (normal length 117) and contains three nucleotides of the 3' untranslated sequence (normal length 278).

The construct contains the complete unmodified Il2 coding sequence. The 3' sequence has been deleted and is replaced by the rabbit beta-globin 3'untranslated sequence and poly A signal contained in the vector. The 3'tail of lymphokine messages is known to destabilize the mRNA. (Figure 5, see Appendix D).

The human Il2 cDNA construct is cloned into the Sal 1 site of the pBMG-neo plasmid.

4.4.2 The Plasmid Carrier. The complete Il2 expression unit consists of the murine metallothioneine promotor, the Il2 cDNA, the second rabbit β-globin intron, and the rabbit β-globin poly A signal. As shown above this expression unit is quite active (5720U/mlx5x10^6 cells x24hrs) in human SCLC upon transfection.

The plasmid carrier is pBMG-Neo. It contains the neomycin resistance gene expressed under the thymidine kinase promotor (for eukaryotic cells) and the Neo^R promotor (for prokaryotes). The thymidine kinase poly A signal is used as termination signal (Figure 6, see Appendix D). For more efficient eukaryotic expression the plasmid contains 2.7kb of the human β-globin sequence. The eukaryotic replicating unit consist of a 69% fragment of the bovine papilloma virus 1 (BPV1). (Figure 7 see Appendix D; Figure 8, see Appendix D). The L1 and L2 sequences coding for the major structural proteins have been deleted. The 69% fragment contains the E1 to E8 early genes and regulatory sequences including the origin of replication. For eukaryotic replication E1 and E2 are absolutely required and are responsible for the episomal replication as nuclear plasmid (85). The copy number is controlled by the cell type and replication appears to occur in synchrony with cell division.

The E6 and E7 gene products of some human papilloma subtypes (HPV16, 18) have been shown to interact the Rb protein and p53, respectively (86). E5 of BPV1 activates EGF receptors in the absence extraneous growth factors (87).

The final piece of pBMG-Neo is a fragment of pBR322 containing the origin of replication for E. coli and the ampicillin resistance gene.

4.4.3 Literature: Evidence against replication competence of the 69% BPV

Is the 69% bovine papilloma virus fragment replication defective for infectious virus particles?

A review of the older literature (refs 88-95) indicates that papilloma viruses in general cannot be produced in tissue culture systems despite many attempts. The reason for this is the fact that the expression of the capsid proteins L1 and L2 and virus production seem to occur only in terminally differentiated epithelial cells that do not grow in culture. A typical quotation states, " The virus cannot be propagated in cell culture and studies at the molecular level require application of its DNA in bacteria" (89). Live virus has been generated only from the extraction from warts or fibro papillomas (formed *in vivo*). Thus, since the intact virus can not be generated *in vitro*, it will also be impossible to generate an incomplete virus lacking both major structural proteins (L1, L2). Direct assays in this direction are therefore futile.

That the L1 and L2 protein (missing in pBMG-Neo) are essential for viral assembly is evident from a recent publication (Kirnbauer et al. 1992, ref 96) demonstrating that the L1 capsid protein (produced by recombinant DNA techniques) on its own is sufficient for assembly of virus-like particles. L1 of BPV1 forms pentamers which assemble into 72 pentameric capsomeres, the same organization seen in wild type virus extracted from bovine warts. It seems evident that a viral DNA lacking L1 and L2 will not be able to produce functional viral particles.

The L1 protein is highly immunogenic and generates a neutralizing antibody response which would also decrease the risk of viral spread (96).

4.4.4 Discussion of risk assessment of the Vector System:

Plasmid BMG-neo is derived from the bovine papilloma virus, which is a transforming virus in some cells. For the purpose of this project the following points are relevant:

1. Tumor cells are being transfected with lymphokine cDNA. Tumor cells are transformed by definition and the risk of further transformation by the plasmid appears insignificant. Growth rate comparisons and morphology of untransfected and transfected tumor cells showed no difference for murine LLC and human SCLC.

2. The multicopy number (20-100/cell) of the plasmid is an important advantage of pBMG-Neo (see preliminary results). It allows very high levels of lymphokine cDNA expression which may be important for the induction of an immune response. The multicopy plasmid in addition allows continued production of lymphokine even after lethal irradiation of the tumor cell.

3. Tumor cells will be lethally irradiated prior to injection precluding their proliferation at the injection site (see chapter 4.6.2). Lymphokine production, will mediate a cellular immune response. This response will make it unlikely that any of the cells escaping radiation will survive.

4. No risk of virus production is apparent since only a viral fragment is used and since helper virus is unnecessary for packaging (see also next chapter 4.4.5).

5. No risk of insertional mutagenesis, since the plasmid replicates extrachromosomally as episome at a copy number of 20-100 per cell (depending on the cell type).

6. Animal papilloma viruses are generally species specific (4), further reducing the risk of viral spread (see also 4.4.5).

7. Stable and high levels expression of cDNAs under the murine metallothionein promotor is regularly achieved in human and other mammalian cells most likely due to the intermediate plasmid copy number. Generally, 50-100 fold more protein can be expressed than with single copy vectors (5,6). High level and stable expression may be important for the success of gene therapy.

8. The plasmid contains a selectable marker for neomycin resistance and has been constructed to allow expression of one or two cDNAs simultaneously. The use of the vector is not limited by size restrictions of inserted DNA.

4.4.5 Literature: Evidence against viral rescue or recombination:
Can human papilloma viruses rescue a bovine, incomplete virus?

In principle the possibility cannot be denied. There is however extensive evidence that such an event is exceedingly unlikely and maybe not possible *in vivo*. In the following the evidence for this statement is cited:

a) complete virus particles have only been observed in stratified squamous epithelial cells, *in vivo*. Other cell types have never been seen to harbor virus particles or produce virus *in vitro* (see above). For virus particles to form, L1 and L2 must be expressed. This occurs only in terminally differentiated epithelial cells. Small cell lung carcinoma cells are neuroendocrine cells. They are not likely to harbor virus or to be able to produce virus, even if they contained the complete BPV1 or a recombined genome.

b) Papilloma viruses are extremely species specific. Never has virus production been observed in a heterologous host. BPV1 can only be produced from bovine warts. Even though BPV1 can cause tumors in hamsters (non epithelial tissues), hamsters will not produce BPV1 particles. Many laboratories have attempted to produce papilloma virus particles in heterologous hosts without success. It is therefore almost inconceivable that BPV1 could be produced in human host. Studies of slaughterhouse butchers' warts have not revealed any unusual distribution of viral subtypes or any evidence for BPV-HPV recombination, even though the incidence of warts in one slaughterhouse, using less automated techniques, was increased (97).

c) Many attempts have been made at pseudotyping papillomaviruses; again all attempts were without success. Even in papilloma viruses within the same species recombination has not been observed. It is not uncommon for humans and other species to harbor more than one papilloma subtype.

In no case has recombination between subtypes been observed. Reports exist of several types of HPV virus within the same lesion; however recombination could not be documented. Finally, in Epidermodysplasia verruciformis many human papilloma virus subtypes have been observed in the same individuals. Again no single event of recombination could be documented (98, 99). Pseudotypes (generated by recombination) are unknown in the field of papilloma virus despite extensive search. Thus a recombination between bovine and human virus appears exceedingly unlikely.

d) The pBMG-Neo vector containing IL2 contains 14.8kb DNA. Even though the packaging requirements for papilloma viruses are not well understood, the structure of the assembled L1 capsid protein (ref 96) would appear to make packaging requirements relatively stringent. It is unlikely that the 14.8 kp BPV1 plasmid plus recombined L1 and L2 (for the hypothetical argument of recombination) could meet the packaging requirements, having twice the genome size of the natural virus; the genome size of the complete BPV1 is 8kb.

Given the constraints of host specificity and of virus production *in vitro* and the lack of any observed and documented recombination *in vivo* and *in vitro*, it is not practical to devise screening assays for events that have never been observed despite intensive search. This is tantamount to saying that the risk of virus production is minimal (or non existent) and cannot be determined with current assays.

What then is the best way of guarding against this minimal remaining risk?

We propose to screen all cells to be transfected by Southern blotting for human papilloma viruses. This will eliminate the majority of occurrences of HPV infection and further minimize the minimal risk of bovine-human PV recombination.

4.4.6 Precautions:

Precautions against recombination and formation of a hybrid human bovine virus:
Homology comparison of BPV1 with human papilloma virus (HPV) sequences in the genbank data base indicates HPV 6 and 31 as the human subtypes with the highest homology to BPV1 (Figure 9; see Appendix D). Inspection of the aligned sequences shows, despite similar overall organization, only relatively limited homology. The longest stretch of uninterrupted sequence identity corresponds to 14 nucleotides, probably insufficient to support homologous recombination. Papilloma viruses are highly species specific (4). Therefore even in the event of a homologous recombination and generation of a hybrid virus it is highly questionable whether such a virus would be infectious for human cells.

Nonetheless we are taking the following precautions. Human tumor cells to be transfected will be first screened by Southern dot blots for the presence of human papilloma virus sequences. This procedure will cover HPV type 6, 11, 16, 18, 31, 33, 35 which encompass the majority of all occurrences. Positive cells will not be used for transfection.

In our studies pBMG-Neo will be used to transfect tumor cells for the expression of lymphokines. Tumor cells are fully transformed cells. Transfection of murine and human tumor cells does not decrease the doubling time (Table 1, see Appendix C) and transfected cells do not appear to be more transformed than untransfected cells in phenotype and morphology. As precaution against tumor growth at the injection site, we are planning to irradiate tumor cells in such a way that their replication is impaired, but that they continue to produce the transferred gene products (lymphokines). The occasional surviving cell even after irradiation is expected to be eliminated by the ensuing immune response at the injection site due to lymphokine production.

The potential benefit of an induced immune response in our opinion by far outweighs the small risk of surviving tumor cells.

4.5 Transfection of SCLC

Plasmid BMG-Neo is a member of the newest generation of expression vectors (44) that affords selection of transfected cells by G418 through its content of a Neomycin resistance cassette transcribed under the influence of the thymidine kinase promotor. The cDNA of interest is expressed under the ubiquitously active metallothionein promotor. Most important for high level and stable expression is the multicopy number of the plasmid (20-100 per cell) which is maintained as an episome in the cell (45). The latter property derives from the 5.5kb viral (69%) fragment derived from the bovine papilloma virus type 1. Finally the plasmid has the pBR322 origin of replication and ampicillin resistance for maintenance in E. coli.

A bottleneck in the expression of eukaryotic genes often is the difficulty to establish the DNA to be expressed in the appropriate cell. One way to achieve this was through the development of retroviral vectors. By generation of infectious but unproductive viruses with the aid of a helper virus containing (packaging) line, DNA is transferred to cells by the broad specificity of the virus particle. Elegant though this technique is, it is limited by the size of the inserted DNA, by the inability to control the locus of insertion into the genome with its potentially negative consequences such as low level expression, and by the small but worrying risk of generating complete infectious retroviruses during the packaging process.

To avoid the use of infectious virus as a DNA transfer vehicle, we have used the Lipofectin technique, which also was successful in the murine LLC model. To date we have transfected 4 SCLC lines previously established by Dr. Savaraj. Since the doubling time of these cells is usually around 30h, a minimum period of two to three weeks is required to establish neomycin resistant lines. The same period again is required to expand the cells to sufficient numbers to be used for therapy. Monitoring of the growth rate and morphology of transfected cells showed no change in comparison to untransfected cells during the time span monitored (2 months). The transfected cell lines tested for biological activity produce the expected product. Moreover, the levels produced are quite high (> 1000U Il2 in 24h by 10^6 cells) and will certainly be sufficient to induce a vigorous immune response upon immunization of patients.

In the Lipofectin technique, $5x10^6$ SCLC are washed twice with serum free medium (Opti-MEM, Gibco) and plated in 6cm tissue culture petri dishes (Falcon 3002) in 3ml OPT1-MEM. The Lipofectin DNA complex is generated by 5 min incubation at room temperature of 10 μg plasmid DNA with 50μg Lipofectin in a volume of 100μl H_2O and then slowly added with swirling to the cell culture. The cells are incubated without further additions in the CO2 incubator overnight and then an equal volume of culture medium (IMDMEM) containing 20% FCS is added. After 48h 1 mg/ml G418 geneticin is added. The culture medium is changed and replaced with fresh G418 every two days until new colonies start growing. Then the medium is changed twice per week. The cells are expanded by continuous culture in standard medium [10% FCS, Iscove's modified Dulbecco's minimum essential medium (IMDMEM)], certified free of contaminants. For mycoplasma detection prior to patient treatment, samples are sent to the American Type Culture Collection (ATCC), for culture and staining. Il2 production in murine and human cells is summarized in Tables 8 and 9, Appendix C. Typically, both murine LLC and human SCLC produce a total of 1,000 to 2,000 U Il2 in 24h when cells are 1-$5x10^6$/ml. This is sufficient to result in tumor rejection in mice.

4.6 Other Safety Considerations

Prior to transfection the established cell lines will be tested for the content of human papilloma virus (HPV) sequences. Cells that are positive for HPV will be excluded from further studies. After selection for neomycin resistance in G418 the cells will be expanded and tested for the production of the transfected lymphokine by standard biological assays.

Transfected cells for use in patients will be screened for contamination as indicated below.

4.6.1 Papilloma Virus Tests

Papilloma virus tests will be performed to detect human papilloma virus sequences which could possibly interact with the bovine papilloma virus component of the transfecting vector pBMG Neo possibly resulting in a promoting effect on human papilloma infections. The ViraType HPV DNA Typing Kit is based on the specific binding of complementary ^{32}P-labeled RNA probes to target DNA from infected cells. Viral DNA released from disrupted cells is denatured and bound to a solid support by filtration through nylon membranes having a high affinity for nucleic acids. Three replicate membranes are prepared by filtration of an aliquot of the sample onto each of three membranes. HPV target DNA bound to the three replicate membranes is then hybridized to three distinct probe groups specific for HPV types 6/11, 16/18 or 31/33/35. The ViraType HPV DNA Typing Kit employs ^{32}P-labeled RNA probes prepared by *in vitro* transcription of recombinant plasmids containing substantially the entire DNA sequences of HPV types 6, 11, 16, 18, 31, 33, and 35. Following hybridization, the nylon membranes are treated with ribonuclease to remove unhybridized probe and washed under

conditions which allow only highly contemporary sequences of probe to remain bound to target DNA. The presence of specifically bound ^{32}P-labeled RNA probe is detected by autoradiography of the nylon membranes. The inherent specificity of nucleic acid hybridization is enhanced in this assay by the use of RNA probes. This approach allows an enzymatic digestion step to reduce nonspecific binding of probes, thus minimizing background signal. In addition, the performance of separate hybridizations with three discrete probe mixtures provides an in there control for nonspecific hybridization for each specimen. It is stated that approximately 70% of all HPV infected patients contain one or more of the HPV strains detectable by our assay.

4.6.2 Il2 production of irradiated cells.

Prior to injection the transfected, Il2 producing SCLC cells will be irradiated with 12,000 rad. Table 10a, b, Appendix C shows that irradiated SCLC, similar to the murine LLC, continue to produce Il2 for up to 12 days. Thus, even though the cells are not replication competent, they still function to produce lymphokine and may induce a strong immune response.

4.6.3 Clonogenic assay of irradiated cells:

To ascertain the absence of surviving cells post irradiation (12,000 rad) of the Il2 producing pBMG-Neo-Il2 transfected SCLC lines clonogenic assays were carried out in the absence or presence of feeder cells (Table 10a, b, see Appendix C). The cloning efficiency of two SCLC lines (non irradiated) measured in the presence of 10,000 irradiated feeder cells, plated in a volume of 0.2ml in microtiter plates, is 58 and 46%. In the absence of feeder cells, plating efficiency at 1 cell per well is 0 and 2%.

Plating irradiated cells (12,000 rad) at 10^4 cells/well, no colony was detected after three weeks, which is ample time for colonies to form. A total of $15x10^6$ cells were plated in three separate experiments as described, and no colonies were detected. Allowing for the plating efficiency as determined above in the presence of irradiated feeder cells our data indicates that there are fewer than 1 live cells in $6.8x10^6$ irradiated SCLC. It appears therefore that the injection of $5x10^6$ irradiated cells for patient treatment is safe and that tumor outgrowth is extremely unlikely.

4.6.4 Microbacterial Studies

Microbacterial studies include fungal cultures, aerobic and anaerobic cultures for bacteria and screening for mycoplasma.

4.6.5 Endotoxin Assay

Endotoxin assay by the Limulus amoebocyte lysate assay.

4.7 GENE THERAPY

Not sooner than two months after the completion of chemotherapy, patients who continue to satisfy the eligibility criteria previously listed and who have cell lines with adequate numbers of cells stably transfected with interleukin genes will receive these transfected cells after all biosafety assessments required by the IRB, FDA and RAC- approved protocol have been completed. The gene-transfected cells will administered subcutaneously under the skin of the forearm.

NOTE: All cells will be irradiated immediately before administration to the patient to prevent their subsequent replication. Our preliminary studies indicate that the dose of irradiation that prevents replication of cells while not impairing their ability to produce interleukin-2 is 6,000 cGy.

A dose of 5 x 10^6 cells will be administered initially. To allow time for development of an immune response, the second dose will be given on day 14; four subsequent doses will be given weekly and the patients observed for response. In responding patients, therapy with gene transfected cells will be repeated in courses consisting of 6 weekly injections at 1-2 month intervals.

The initial treatment with transfected cancer cells will be given in the University of Miami's Clinical Research Center. Patients will be hospitalized for 24 hours after administration to ensure careful monitoring for any unexpected side effects in a setting where third party payor reimbursement is not an issue. During the course of therapy, patients will undergo serial assessment of the clinical status of their disease status as defined below.

In addition, peripheral blood samples will be obtained weekly for analysis of the mononuclear cell fraction for the presence of tumor specific T effector cells, by phenotypic and functional assays. In vitro studies for lymphocyte reactivity and lytic activity will be performed, using tumor cells as targets. Other reagent target cells will include K562 and Daudi for NK and LAK cells, the patient's cancer cells cryopreserved from the initial biopsy (if available in adequate number), and the cancer cells from the established lines of the patient and other patients, to determine specificity for autologous and allogeneic targets. The patients' cell lines pre- and post-transfection will be assayed for expression of HLA class I and II antigens to determine whether expression of these antigens correlates with immune responsiveness and whether gene transfection with Il-2 alters their expression.

NOTE: We recognize that the irradiated tumor cells will only survive 7-10 days in vivo; the time course of their Il-2 production is therefore limited. The number of activated T effector cells may therefore be small and difficult to detect by the above studies because of the dilution in large numbers of unaltered circulating lymphocytes. Other laboratory projects at our center, however, intend to expand the number of these effector cells in vitro and to clone individual subsets of killer cells. The yield from these experiments will enhance our ability to assess changes in the patients' immune reactivity to their own cancer cells. Moreover, the clonally expanded cells will be available as reagents for gene transfection studies.

5.0 TOXICITY ASSESSMENT AND REPORTING

The patients will be carefully evaluated over the first 24 hours after administration of the transfected cells, and then will be evaluated weekly until gene therapy terminates. Thereafter for study purposes, the patient will be followed monthly clinically to monitor disease status and survival.

Patients will be observed for any side effects, which will be graded based on the Eastern Cooperative Oncology Group (ECOG) Toxicity Rating Scale, which grades toxicity as 0 (none), 1 (mild), 2 (moderate), 3 (severe), 4 (life-threatening), and 5 (lethal).

We do not expect significant side effects from this study because a relatively small volume of tumor cells will be administered, the tumor cells will not be replicating, only a short base sequence of viral (papilloma) replicase is utilized in our gene insertion technique, patients with active papilloma virus infection (warts) are excluded, and the amount of Il-2 produced locally by the subcutaneously administered transfected tumor cells is unlikely to have systemic effects. We will nevertheless monitor patients for the development of side effects that have been reported to occur after lymphokine administration, including the following: malaise; fever; nausea and/or vomiting; diarrhea; dyspnea;

eosinophilia; anemia; capillary leak syndrome with fluid retention, weight gain, congestive heart failure, and hypotension; pruritus; transient liver and renal function abnormalities; nasal congestion and glossitis; mental status changes; rash; thrombocytopenia; oliguria; hair loss; myocardial infarction and cardiac arrhythmias; arthritis; and visual abnormalities.

Any previously unknown ECOG grade 2 and 3 adverse reactions will be reported in writing within 10 working days to our IRB, the FDA, and the NCI.

Any grade 4 adverse reaction or patient death while on gene therapy treatment will be reported to our IRB, the FDA, and the NCI by phone within 24 hours, with a written report to follow within 10 working days.

6.0 CRITERIA FOR CLINICAL EVALUATION AND DEFINITION OF RESPONSE

Complete Remission: Complete disappearance of all measurable and evaluable disease. No new lesions. No disease-related symptoms. No evidence of non-evaluable disease, including normalization of abnormal laboratory values. Status must be maintained for one month to qualify.

Partial Remission: Greater than or equal to a 50% decrease from baseline in the sum of products of perpendicular diameters of all measurable lesions. No progression of evaluable, but non-measurable disease. No new lesions. Status must be maintained for one month to qualify.

Stable Disease/No Response: Disease status not defined by criteria for Complete or Partial Remission (above) or Progressive Disease (below).

Progressive Disease: 50% increase in the sum of the products of measurable lesions over the smallest sum observed (or over baseline status if no intervening decrease), or reappearance of a lesion which had disappeared, or worsening of evaluable, non-measurable disease, or appearance of any new lesion/site.

7.0 STUDY PARAMETERS

Test/Eval	Before ChemoRx	During ChemoRX	Before GeneRX	During GeneRX	After GeneRx
History, PE, PS	X	Q 3 wks	X	Weekly	Monthly
CBC,Diff,Plts	X	Weekly	X	Weekly	Monthly
ALT,AST,LDH,Alk. P'ase,Bilirubin	X	Q 3 wks	X	Weekly	Monthly
Creat.,Uric Acid	X	Q 3 wks	X	Weekly	Monthly
Na,K,Cl,Calcium	X	Q 3wks	X	Weekly	Monthly
Chest X-ray	X	Q 3 wks	X	Monthly	Monthly
Bone Scan	X				
CT of Brain	X				
CT of Chest	X	at end	X	at end	prn
CT of Abdomen	X	at end	X	prn	prn
MUGA SCAN [1]	X				

Test/Eval	Before ChemoRx	During ChemoRX	Before GeneRX	During GeneRX	After GeneRx
HIV assay	X				
Pregnancy Test	X				
Marrow Asp./Bx	X				
Lymph Node Bx	X				
Peripheral Blood Sampling [2]			X	Q 2 wks	Monthly

[1] Required for patients with a prior history of cardiac disease.

[2] 40 ml of peripheral blood will be obtained for phenotypic and functional characterization of activated killer cells and to initiate cloning of subsets of killer cells. Samples will be obtained before, and every two weeks x4 during courses of gene therapy, and then monthly.

Dr. Michael Kolber is conducting animal studies of the accelerated clearance of locally implanted radiolabeled transfected tumor cells as a surrogate marker for the generation of a cytocidal immune response. If the results of the initial studies are borne out by ongoing experiments in mice, we will employ this technique in our human studies. The initial injection of transfected tumor cells will be Indium labeled. Surface counts over the site of the forearm injection will be obtained by a gamma counter probe three times/week for the next two weeks to generate a curve of isotopic clearance. A subsequent injection using radiolabeled cells will be similarly evaluated to determine whether the transfected tumor cells injected later undergo more rapid clearance than the initially injected cells did. Correlation of these data with the results of in vitro tests of immunologic reactivity will be sought to confirm the value of this radiolabeled approach as a measure of immunologic response to transfected tumor cells.

8.0 STATISTICAL CONSIDERATIONS

The results of this approach to engineering an immune response to cancer will be evaluated clinically and in the laboratory.

8.1 Clinical Response to Therapy

At the time of treatment with gene transfected cells, patients will either be in complete or partial remission from therapy or will be relapsing from a previous complete or partial remission. The outcome for each group of such patients with conventional treatment is fairly predictable in a narrow range, in terms of progression-free and overall survival. If the gene transfected patients show a $\geq$ 50% prolongation of the intervals indicated below we will have highly suggestive evidence of a response.

With conventional therapy, the approximate length of progression-free and overall survival for patients with limited stage disease based on disease status is as follows:

	Median Survival	Progression-Free Survival
Complete Remission	16 months	12 months
Partial Remission	12 months	9 months
Relapsing	3-4 months	---

8.2 Laboratory Measures of Response to Therapy

Weekly samples of peripheral blood samples will be analyzed for the presence of tumor specific cytotoxic T-cells as described in Section 4.7 to obtain an assessment of biologic response and for correlation with clinical response.

8.3 Safety Considerations

The sample size requirement for this study is driven in part by the need to monitor for toxicity and unexpected effects. We will treat 12 patients with LD SCLC with Il-2 transfected tumor cells. This will provide an 80% probability of finding at least one episode of any adverse event that occurs with a true frequency of at least 13%, and a 90% chance if the true frequency is 17%.

8.4 Accrual of Patients

Using I1-2 transfected tumor cells, we will treat 12 patients with LD SCLC within two years. In order to provide 12 patients who are eligible and evaluable for gene therapy approximately 40 patients over two years will have to be entered on study because of the following considerations.

The proportion of patients with limited disease SCLC:

- who satisfy eligibility criteria, especially good performance status and absence of adverse prognostic factors is approximately 80%.
- whose tumor cells can be established as cell lines in culture is approximately 70% (from Dr. Savaraj's experience).
- whose cell lines can be stably transfected with the gene for Il-2 production is approximately 90% (from Dr. Podack's experience).
- who will achieve a complete or good partial remission with chemotherapy is approximately 80%.
- who will relapse and require therapy or for a variety of medical and non-medical reasons will become ineligible prior to gene therapy is approximately 25%.

Assuming that these conservative estimates are all independent variables, then the proportion of entering patients who will be eligible for gene therapy is $0.8 \times 0.7 \times 0.9 \times 0.8 \times 0.75 = 0.30$ Since only approximately 3/10 entering patients will undergo subsequent gene therapy, we will need to accrue 40 patients for initial evaluation so that 12 will be available to receive gene therapy. At the University of Miami Medical Center, we care for approximately 60 patients annually who are newly diagnosed with SCLC. Approximately 1/3 (or 20) of these patients are LD stage. We believe our current population will provide an adequate number of patients to meet our accrual goals.

Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene in Limited Stage Small-Cell Lung Cancer.

Patient's Informed Consent Form

Research Study

I, _____ willing agree to participate in this research study which has been explained to me by Dr. _____. This study is being conducted at the University of Miami School of Medicine.

Purpose of the Study

It has been explained to me that I have small-cell cancer of the lung. I have been invited to participate in a research study. I understand that the study is being undertaken in an effort to evaluate the safety and effectiveness of the treatment described below and gain information about my disease. Although the intent is to slow, decrease, or stop the growth of my cancer, I realize that it is impossible to predict whether any benefit will occur from my participation in this study.

Description of Procedures

In this investigative treatment protocol, cancer cells obtained from me by biopsy will be cultured to grow the cells in test tubes. Although these cells may be available from the biopsy taken to make the diagnosis of lung cancer, I understand that a second biopsy may be needed for the purposes of this research study. The second biopsy, if needed, may require only local anesthesia, but could also involve general anesthesia and mediastinoscopy (passage of a tube behind the sternum through an incision at the base of the neck) with its attendant risks, which are described below. Genetic material that instructs cells to produce a substance called Interleukin-2 will be inserted into the cancer cells by means of a gene carrying system called a plasmid. This gene carrying system includes the transfer into the cells of a portion of the bovine papilloma virus 1, a virus that causes tumors in cows. The cells are then cultured for additional time so that they reproduce, making many more cells. These cells will be removed from culture and treated with radiation therapy so that they cannot regrow in my body but will still be alive. They will then be injected under the skin of one of my forearms. These injections will be done initially once, again in two weeks, and then every week for 4-6 weeks thereafter. I will be hospitalized for 24 hours after the initial injection for observation for any immediate side effects. If there is a good response to treatment, it is possible that additional injections may be administered. I understand that the intent is that the tumor cells returned under the skin of my arm will produce Interleukin-2, which is a substance that stimulates the immune system to produce cells which can attack and kill cancer cells. The hope is that the repeated injections will cause my body to make these cancer-cell killers in adequate numbers not only to attack the cancer cells which have been injected under my arm but also to circulate in the blood and destroy other cancer cells that remain in my body. Although this treatment technique has been shown to be effective in lung cancer in mice, this approach has not been used in humans before. Administration of Interleukin-2 alone into a vein in very high doses can stimulate the immune system to destroy some cancers in a small number of patients. The administration of Interleukin-2 in those very high doses has many severe side effects. The amount of Interleukin-2 made by the tumor cells in this study that will be returned to me will be substantially less.

Risks and Discomforts

If an additional biopsy is required, the local or general anesthesia and/or the biopsy itself can rarely cause fatal or serious complications, including bleeding, infection and post-operative pain.

Significant side effects from this treatment approach are not expected for a number of reasons. As mentioned previously, the amount of Interleukin-2 produced locally by the tumor cells that have been injected into my forearm is unlikely to have side effects. I will nevertheless be monitored for the development of side effects that have been reported when Interleukin-2 is administered in high doses, such as fever, nausea and/or vomiting, diarrhea, shortness of breath, anemia, low blood pressure and shock, heart failure, weight gain and fluid retention, itching, abnormalities in liver and kidney function tests, rashes, abnormal blood counts, and visual, mental, and heart rhythm disturbances.

The cancer cells returned to me will be treated with radiation before they are returned to me which should prevent them from reproducing. Nevertheless, there is an unlikely possibility that my cancer cells could grow at the sites of reinjection or that combination of the papilloma virus material with my cancer cells will produce a new kind of cancer locally at the injection sites or which could spread throughout my body. There is a remote possibility that the portion of the genetic material in the tumor cells obtained from a part of the bovine papilloma virus could recombine with human genetic material and lead to active viral infection. It has been explained to me that this is highly unlikely because the papilloma virus of cows is usually specific for cows and does not infect humans and because only a fragment of the whole virus is involved in this gene therapy technique. Nevertheless, because of the potential for infectious virus becoming established, my physicians have strongly urged me to employ contraception throughout this therapy to avoid transmitting virus to a developing fetus. Since this therapy is intended to stimulate my immune system, it is remotely possible that an immune attack on my normal cells and tissues could occur.

My doctor will be watching me closely to see if side effects are occurring. Routine blood and urine tests, scans and x-rays will be done to check the effects of treatment. I understand that in an investigational study of this nature there is always the risk of very uncommon or previously unknown side effects occurring. I understand that my doctor will treat any side effects that occur. I have been told that the institution is not financially responsible for the added cost for treatment of side effects that may occur from this research study.

Contact Persons

For more information concerning the research and research-related risks or injuries, I can notify Dr. _____, the investigator in charge at (305) _____. In addition, I may contact _____ at (305) _____ for information regarding patients' rights in research studies.

Benefits

It is not possible to predict whether or not any personal benefit will result from the use of this treatment program. I have been told that should my disease become worse, side effects become severe, or that my doctor feel that this treatment is no longer in my best interest the treatment will be stopped. Further treatment will be discussed.

Alternatives

Alternatives that could be considered in my case include treatment with chemotherapy that I have received in the past, radiation therapy or different combinations of drugs administered for varying periods of time. An additional alternative is no further therapy. I understand that my doctor can provide detailed information about my disease and the benefits of the various treatments available. I have been told that I should feel free to discuss my disease and my prognosis with the doctor.

I understand that my physician will be available to answer any questions that I may have at any time concerning this study. In addition I understand that I am free to ask my physician any questions concerning this program that I wish to in the future. I should be aware that added costs may arise and some of these costs may not be covered by insurance. My doctor will discuss this with me.

Voluntary Participation

I am aware that my participation in this study is entirely voluntary. No compensation for participation will be given to me. I also understand that I am free to withdraw my consent to participate in this program at any time without prejudice to my subsequent care. I am free to seek care from a physician of my choice at any time. If I do not take part in or withdraw from this study, I will continue to receive care. In the event that I withdraw from the study I will continue to be followed and clinical data will continue to be collected from my medical records.

Confidentiality

I understand that a record of my progress while on this study will be kept in a confidential form at the University of Miami School of Medicine. The confidentiality of my record will be carefully guarded. During any of their required reviews, representatives of the Food and Drug Administration (FDA) and the National Cancer Institute (NCI) may have access to my medical records which contain my identity. However, no information by which I can be identified will be released or published.

I have read all of the above, asked questions, received answers concerning areas I did not understand and I willingly give my consent to participate in this program. Upon signing this form, I will receive a copy.

_____ _____

(Patient's signature) (Date)

_____ _____

(Witness) (Date)

_____ _____

(Physician) (Date)

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Eckhard R. Podack	Professor

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Göttingen, Germany	Ph.D.	1972	Biochemistry
University of Frankfurt, Germany	M.D.	1968	Medicine
University of Frankfurt, Germany	License to Practice	1970	Intern:Internal Med., Surg., Biochem.

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE:

10/70 - 1/72 Fellow, Department of Biochemistry, University of Gottingen, Germany

2/72 - 7/74 Postdoctoral Fellow, Department of Biochemistry, University of Gottingen, Germany; Chairman, Professor Dr. W. Seubert

8/74 - 6/78 Research Associate, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California; Chairman, Professor Dr. H.J. Muller-Eberhard

7/78 - 6/80 Assistant Member I, Department of Immunology, Research Institute of Scripps Clinic, La Jolla,California

7/80 - 5/84 Assistant Member II, Department of Immunology, Research Institute of Scripps Clinic, La Jolla, California

6/84 - 8/87 Professor of Microbiology & Immunology, Department of Microbiology and Immunology, New York Medical College, Valhalla, New York

6/85 - 8/87 Professor of Medicine, New York Medical College, Valhalla, New York, Professor of Microbiology, Immunology, and Medicine.

9/87 - present Professor Microbiology and Immunology,and Medicine, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, Florida.

2/88 - present Professor of Oncology, Department of Oncology, Comprehensive Cancer Center, University of Miami School of Medicine, Miami, Florida

HONORS AND AWARDS:

Annual Award of the German Diabetes Society for doctoral thesis in Biochemistry, 1973.

Investigatorship by the Deutsche Forschungsgemeinschaft, Germany, August 1974 to November 1976.

Established Investigator American Heart Association, July 1979 to June 1984.

Eckert Lecturer, 1987, Case Western Reserve University, Cleveland, Ohio

Associate Editor and Editor of several books and Scientific Journals.

Visiting Professor, University of California, Las Angeles; Case Western Reserve University, Cleveland Ohio and Cleveland Clinic.

Visiting Professor, National Cancer Center, Tokyo, Japan, Division and Medical Oncology, June 1990 September 1990.

PUBLICATIONS (selected from total of 127):

1. Podack, E.R., Lowrey, D.M., Lichtenheld, M., Olsen, K.J., Aebischer, T., Binder, D., Rupp, F., and Hengartner, H. (1988) Structure, function and expression of murine and human perforin 1 (PI). Immunol. Rev., 103:203-211.
2. Podack, E.R. (1988) Granule-mediated cytolysis of target cells. Ibid, pp. 1-10.
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8. Podack, E.R. (1989) Killer Lymphocytes and How They Kill. In: Current Opinion in Cell Biology, 1:929.
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10. Lichtenheld, M.G. and Podack, E.R. (1989) Structure of the Human Perforin Gene: A Simple Gene Organization with Interesting Potential Regulatory Sequences. J. Immunol., 143,4267-4274.
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12. Podack, E.R., and Lichtenheld, M. (1990) Perforin and the mechanism of lymphocyte mediated cytolysis.In: Current Topics. ed. Toni Claudio, (California: Academic Press), 36:161-175.
13. Asch, E. & Podack, E.R. (1990) Vitronectin Binds to Activated Human Platelets and Plays a Role in Platelet Aggregation. J. Clin. Invest. 85:1372-1378.
14. Podack, E.R. (1990) Expression of Perforin and Granzymes and their Role in Cytolysis. In: Local Immunity, eds. Clemens Sorg, 6:136.
15. Podack, E.R. & Hengartner, H. (1990) Structure of Perforin & Its Role in Cytolysis. Year in Immunol.,6:245.
16. Podack, E.R., Hengartner, H. and Lichtenheld, M.G. (1991) A Central Role of Perforin in Cytolysis? Ann. Rev. Immuno., 9:129-152.
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21. Lu, P., Garcia-Sanz, J.A., Lichtenheld, M.G. and Podack, E.R. (1992) Perforin Expression in Human Peripheral Blood Mononuclear Cells. J. Immunol. 148:3354-3360.
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29. Garcia-Sanz, J.A. and Podack, E.R. (1993) Regulation of Perforin Gene Expression in a T cell Hybrid with Inducible Cytolytic Activity. Eur. J. Immunol. in press.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel, consultants, and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Peter A. Cassileth, M.D.	Professor of Medicine

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Union College, Schenectady, N.Y.	B.A.	1958	English
Columbia University College of Physicians and Surgeons	M.D.	1962	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PREVIOUS EMPLOYMENT/EXPERIENCE:

1962-63 Intern in Medicine, Columbia-Presbyterian Medical Center, N.Y.C.
1963-65 Resident in Medicine, Columbia-Presbyterian Medical Center, N.Y.C.
1965-66 Fellow in Hematology, Columbia-Presbyterian Medical Center, N.Y.C.
1966 Chief Resident in Medicine, Delafield Cancer Hospital, Columbia-Presbyterian Medical Center, N.Y.C.
1968-69 Fellow in Hematology, Columbia-Presbyterian Medical Center, N.Y.C.
1966-68 Hematologist/Oncologist, Ireland Army Hospital, Fort Knox, KY
1969-75 Assistant Professor of Medicine, Department of Medicine, University of Pennsylvania
1975-79 Associate Professor of Medicine, Department of Medicine, University of Pennsylvania
1979-92 Professor of Medicine, Department of Medicine, University of Pennsylvania
1992- Professor of Medicine, Department of Medicine, University of Miami
1992- Chief, Division of Hematology/Oncology, University of Miami
1992- Director, Bone Marrow Transplantation Program, University of Miami

HONORS:
1958 Phi Beta Kappa
1961 Alpha Omega Alpha

SELECTED PUBLICATIONS: (N = 98)

1. Spiers, A.S.D., Moore, D., Cassileth, P.A., Harrington, D.P., Cummings, F.J., Neiman, R.S., Bennett, J.M. and O'Connell, M.J.: Remissions in hairy-cell leukemia with pentostatin (2'-deoxycoformycin). N. Engl. J. Med. 316:825-830, 1987.
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8. Brown, E.A., Talbot, G.H., Provencher, M. and Cassileth, P.A.: Anaerobic bacteremia in patients with acute leukemia. Infect. Control Hosp. Epidemiol. 10:65-69, 1989.
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17. Hochster, H. and Cassileth, P.A.: Fludarabine therapy of non-Hodgkin's Lymphoma. SeminarsOncol.17:63-65,1990.
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21. Cassileth, P.A., Andersen, J., Lazarus, H.M., Kaizer, H., Bloom, E., Elfenbein, G.J., Santos, G.W.,Bennett, J.M., Colvin, O.H. and Oken, M.M.: Autologous bone marrow transplantation in acute myeloid leukemia in first remission: an Eastern Cooperative Oncology Group pilot study. In Autologous Bone Marrow Transplantation: Proceedings of the Fifth International Symposium, University of Nebraska Medical Center, 1991, p. 37-42.
22. Paietta, E., VanNess, B., Bennett, J.M., Pellone, C., Billadeau, D., Cassileth, P.A. and Wiernik, P.H.: Unexpected immunoglobulin light chain gene rearrangements in myeloid antigen acute lymphoid leukemias. Leukemia Res. 15: 149-155, 1991.
23. Kramer, Z.B., Boros, L., Wiernik, P.H., Andersen, J., Bennett, J.M., Cassileth, P., and Oken, M.: 13 cis- retinoic acid in the treatment of elderly patients with acute myeloid leukemia. A phase II ECOG pilot study. Cancer 67: 1484-6, 1991.
24. Hochster, H.S., Kyungmann, K., Green, M.D., Mann, R.B., Neiman, R.S., Oken, M.M., Cassileth, P.A., Harris, J.E., Tormey, D.C. and O'Connell, J.J.: Activity of fludarabine in previously treated non-Hodgkin's low grade lymphoma. Results of an Eastern Cooperatiave Oncology Group study. J. Clin. Oncol. 10:28-32.
25. Nowell, P.C., Kant, J.A., Finan, J.B., Cassileth, P.A., and Hanson, C.A.: Marrow fibrosis associated with a Philadel phia chromosome. Cancer Genet Cytogen, 59:89-92, 1992.
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9309-054 O'Shaughnessy, Joyce; National Institutes of Health, Bethesda, Maryland; *Retroviral Mediated Transfer of the Human Multi-Drug Resistance Gene (MDR-1) into Hematopoietic Stem Cells During Autologous Transplantation after Intensive Chemotherapy for Breast Cancer.*

Date of RAC Approval: 9/9/1993
Date of NIH Approval: 10/7/1993

Retroviral Mediated Transfer of the Human Multidrug Resistance Gene (MDR-1) into Hematopoietic Stem Cells during Autologous Transplantation after Intensive Chemotherapy for Breast Cancer

Patients with metastatic breast cancer will receive 4-5 cycles of induction chemotherapy on one of the ongoing Medicine Branch protocols. Patients achieving at least a partial response, and who do not have evidence of bone marrow involvement, will undergo PBSC and bone marrow harvest when hematologic recovery has occurred. Patients who have not achieved a PR, but who are responding to therapy, may be treated with additional cycles of therapy in an attempt to achieve a PR. Such patients will be eligible for transplant if a PR is obtained. 70% of the bone marrow and PBSC will be cryopreserved. The CD34+ subpopulation from the remaining 30% of the bone marrow and PBSC harvest will be obtained using an anti-CD34+ antibody and immunoabsorption column. The bone marrow and peripheral blood CD34 cells will be transduced with a retroviral vector expressing the human MDR-1 cDNA. Patients with positive bone scans or histologic evidence of bone marrow involvement will be excluded from the gene transfer component of the protocol. The MDR-1 transduced CD34 cells will be reinfused along with the non-transduced bone marrow and PBSC into patients following high dose ICE chemotherapy. Serial peripheral blood and bone marrow samples will be obtained to study hematopoietic reconstitution with MDR-1 transduced cells. Patients with residual or progressive disease after ABMT will be treated with taxol or vinblastine. In these relapsed patients, peripheral blood and bone marrow samples will be obtained to study whether chemotherapy amplifies the proportion of hematopoietic cells containing the MDR-1 provirus. We will monitor the nadir blood counts of each patient receiving salvage chemotherapy for evidence of myeloprotection and correlate this data with changes in the mean proviral copy number. Sites of relapsed tumor will be biopsied to test for the presence of the MDR-1 provirus.

Non-Technical Abstract

Autologous Bone Marrow Transplantation (ABMT) is a method of treating breast cancer by giving patients very high doses of chemotherapy and then rescuing them with their bone marrow cells that have been removed and frozen. In this study, patients will be treated initially with standard doses of chemotherapy to cause a remission of their breast cancer. Then, blood cells which are capable of rebuilding patients' bone marrows will be removed from the patients' bloodstream and bone marrow. Seventy percent of these blood cells will be frozen directly and 30% will be used to separate the "stem" cells, those cells thought to be the forebearers of all other blood cells. These stem cells will be cultured with a disabled virus which carries genetic material referred to as the multidrug resistance gene (MDR-1). The virus transfers the MDR-1 gene into a portion of the patient's stem cells. The purpose of putting the MDR-1 gene into the patients' stem cells is to try to make these blood cells and their offspring resistant to the toxic effects of chemotherapy. The MDR-1 protein (Pgp) that is made from the MDR-1 gene makes cells resistant to chemotherapy by pumping the drug out of cells before the drug is able to kill the cell.

Patients are then treated with very high doses of ICE (ifosfamide, carboplatin, and etoposide) chemotherapy and then the frozen blood cells as well as the MDR-1 blood cells are given back to the patient through a catheter in a vein. It is hoped that the MDR-1 stem cells will contribute to the rebuilding of patients' bone marrows following the high-dose chemotherapy. Samples of patients' bone marrows and peripheral blood cells will be obtained at several points after the bone marrow recovers to follow the course and life span of the cells containing the MDR-1 virus. Patients who still have evidence of breast cancer or whose breast cancer returns after ABMT will be treated with taxol, a chemotherapy drug that is pumped out of cells by the MDR-1 protein. We will again follow patients' blood counts closely to determine whether the number of blood cells that contain the MDR-1 gene increases in response to the chemotherapy. Patients will be treated with taxol for as long as there is evidence of a beneficial effect against their tumor.

July 14, 1993

Protocol Numbers:
NCI:T92-0000
Medicine Branch #:MB_ _ _
Clinical Center #:

IND's:
G-CSF: #BB2704
Taxol: #22850
Carboplatin: #19314

Retroviral Mediated Transfer of the Human Multidrug Resistance Gene (MDR-1) into Hematopoietic Stem Cells during Autologous Transplantation after Intensive Chemotherapy for Metastatic Breast Cancer

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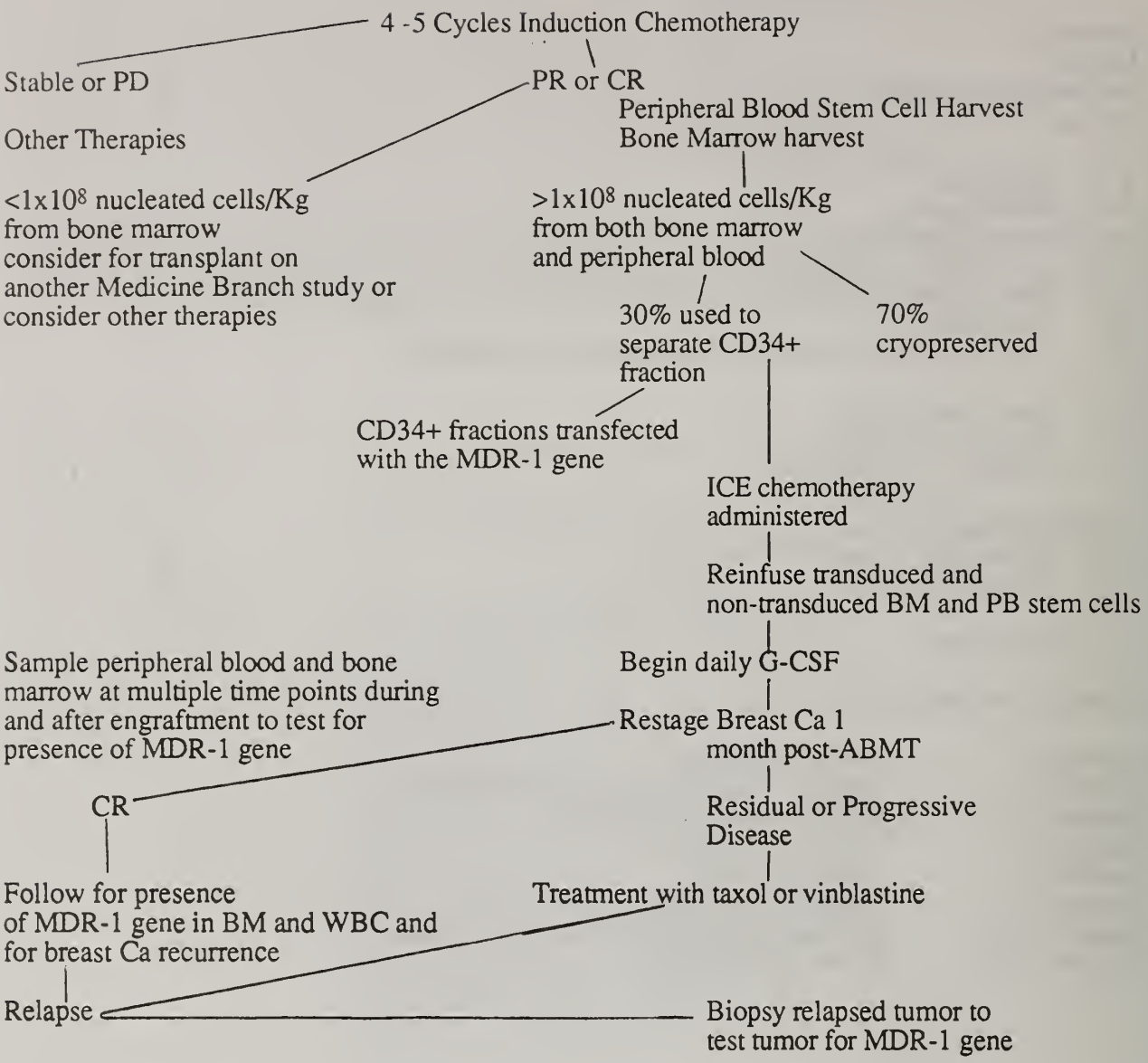
Schema


4 -5 Cycles Induction Chemotherapy
Stable or PD
Other Therapies
<1x10⁸ nucleated cells/Kg from bone marrow consider for transplant on another Medicine Branch study or consider other therapies
PR or CR
Peripheral Blood Stem Cell Harvest
Bone Marrow harvest
>1x10⁸ nucleated cells/Kg from both bone marrow and peripheral blood
30% used to separate CD34+ fraction
70% cryopreserved
CD34+ fractions transfected with the MDR-1 gene
ICE chemotherapy administered
Reinfuse transduced and non-transduced BM and PB stem cells
Begin daily G-CSF
Restage Breast Ca 1 month post-ABMT
Sample peripheral blood and bone marrow at multiple time points during and after engraftment to test for presence of MDR-1 gene
CR
Residual or Progressive Disease
Follow for presence of MDR-1 gene in BM and WBC and for breast Ca recurrence
Treatment with taxol or vinblastine
Relapse
Biopsy relapsed tumor to test tumor for MDR-1 gene

Retroviral Mediated Transfer of the Human Multidrug Resistance Gene (MDR-1) into Hematopoietic Stem Cells during Autologous Transplantation after Intensive Chemotherapy for Breast Cancer

1.0 Objectives

1.1 To study the feasibility of obtaining engraftment after high dose chemotherapy with retrovirally transduced hematopoietic stem cells expressing a cDNA for the human multi-drug resistance-1 (MDR-1) gene.

1.2 To determine whether the transduced MDR-1 gene will confer drug resistance to hematopoietic cells and act as a in vivo dominant selectable marker. This will be defined as amplification of the transduced hematopoietic cell population in patients receiving post-transplantation chemotherapy.

2.0 Background

Stage IV breast cancer is currently incurable. The median survival for patients with metastatic disease is 18-24 months. Both hormonal and chemotherapy are considered palliative and have not been definitively shown to improve either disease-free (DFS) or overall survival (OS) (1).

Because many chemotherapy drugs have steep tumoricidal dose-response curves, a substantial increase in cell kill may be achieved by significantly increasing the dose. Bone marrow suppression is a major dose limiting toxicity of intensive chemotherapy. The use of autologous peripheral blood and/or bone marrow cells combined with administration of recombinant hematopoietic growth factors restores bone marrow function, thereby allowing dose-escalation over conventional chemotherapy. Studies of high dose chemotherapy and autologous bone marrow transplantation (ABMT) following 3-4 cycles of induction therapy in chemotherapy responsive breast cancer have shown very high complete response rates of 50% (2-4). While the median time to treatment failure and median survival appear to be no better than that achieved with conventional chemotherapy, three trials have reported that 15-20% of all patients undergoing ABMT are alive and disease-free at 3 years (2-4). This compares favorably with the results of conventional chemotherapy where fewer than 10% of patients are generally disease-free at 3 years (5,6). Many patients will relapse following ABMT, and bone marrow suppression remains as a major dose-limiting complication of post-transplantation chemotherapy. New approaches are needed to circumvent the bone marrow toxicity associated with high dose chemotherapy.

One approach would be to make normal bone marrow cells resistant to the toxic effects of chemotherapy. This might be accomplished by introducing specific drug resistance genes into hematopoietic cells. Retroviral mediated gene transfer of the dihydrofolate reductase gene into murine hematopoietic stem cells resulted in relative resistance to the myelosuppressive effects of the antineoplastic drug methotrexate (7). Another candidate drug resistance gene is the human MDR-1 gene which encodes for a 170-kilodalton transmembrane glycoprotein called P-glycoprotein (Pgp). Pgp expression in primary tumor cells confers pleiotropic resistance to a number of naturally occurring chemotherapeutic agents by enhancing their elimination from the cell (8,9). Transfer of the MDR-1 gene to protect the bone marrow cells of breast cancer patients is attractive because several drugs with clinical activity in the treatment of breast cancer, including taxol and vinblastine, are effluxed by Pgp (10).

The endogenous MDR-1 gene is expressed in primitive hematopoietic cells at low levels, and expression is extinguished with myeloid maturation (11-13). Transgenic mice in which the human MDR-1 cDNA is constitutively expressed in hematopoietic cells acquire resistance to the myelosuppressive effects of chemotherapeutic drugs (10). Drug sensitive mice can be

rendered resistant to chemotherapeutic myelosuppression following transplantation of bone marrow from MDR-1 transgenic mice (14).

Our work in a murine model has established that retroviral gene transfer may be used to introduce and express the MDR-1 cDNA in hematopoietic cells (15). Similar results have been reported by others (16). The level of expression of the retrovirally transduced human MDR-1 cDNA in primary hematopoietic cells is substantially higher than the level of expression from the endogenous murine multidrug resistance genes mdr-1 and mdr-3 (B. Sorrentino, unpublished observations, Appendix I). Furthermore, we have shown that the MDR-1 cDNA confers preferential survival on retrovirally transduced hematopoietic cells following treatment with the chemotherapeutic drug taxol (15). Preferential survival may be traced to retrovirally transduced, long-lived primitive hematopoietic cells that can be transplanted to secondary recipients (B. Sorrentino, unpublished observations, Appendix I).

Patients with Stage IV breast cancer are currently treated with high dose ifosfamide, carboplatin, and etoposide (ICE) followed by autologous bone marrow transplantation (ABMT) on active NCI Medicine Branch protocols at NIH. Patients requiring further treatment after ABMT have been treated with taxol and vinblastine on other protocols. Taxol is an active drug in breast cancer with a 56 percent response rate in previously treated metastatic breast cancer (17). Vinblastine has a 20-30 percent response rate in the salvage therapy of breast cancer (18).

We propose to use retroviral gene transfer to introduce the MDR-1 cDNA into the hematopoietic cells of these patients who are receiving ICE chemotherapy followed by ABMT. These experiments will allow us to address two basic questions. First, the feasibility of introducing and expressing drug-resistance genes in the bone marrow of patients receiving chemotherapy for cancer can be explored. Second, in the patients requiring post-transplant therapy with taxol or vinblastine, to test if the MDR-1 gene can be used as a dominant selectable marker in vivo and therefore potentially overcome current limitations in gene transfer efficiency into hematopoietic stem cells. Based on our pre-clinical studies, we anticipate that transduced hematopoietic cells constitutively expressing retrovirally encoded Pgp will have a survival advantage over non-transduced cells following chemotherapy. This will be reflected in an increase in the proportion of hematopoietic cells carrying and expressing the proviral MDR-1 cDNA. If significant enrichment of MDR-1 expressing cells occurs, it is possible that patients' bone marrow will become more resistant to the myelosuppressive effects of chemotherapy. We will monitor the nadir blood counts of each patient receiving salvage chemotherapy for evidence of myeloprotection and correlate this data with changes in the mean proviral copy number. Preliminary findings suggestive of myeloprotection would need to be confirmed in a later controlled study.

These studies represent the initial steps toward a long-term goal of creating a fully drug resistant bone marrow. If combined with more efficient approaches for transduction of hematopoietic cells, these experiments may contribute to the development of new, intensive chemotherapy regimens for the treatment of malignant disease. In addition, our studies have important potential applications in the selection of genetically modified hematopoietic cells and the treatment of non-malignant disorders.

The design of this study is analogous to our protocol entitled, "Pilot Study of High-Dose ICE (Ifosfamide, Carboplatin, Etoposide) Chemotherapy and Autologous Bone Marrow Transplant (ABMT) with neoR-transduced Bone Marrow Stem Cells" and its Amendment "Genetic Marking with Retroviral Vectors to Study the Feasibility of Stem Cell Gene Transfer and the Biology of Hematopoietic Reconstitution after Autologous Transplantation in Multiple Myeloma, Chronic Myelogenous Leukemia or Metastatic Breast Cancer", which have been approved by the Institutional Review Board (IRB), the Institutional Biosafety Committee

(IBC) and the Recombinant DNA Advisory Committee (RAC). These protocols have been implemented and we are actively enrolling patients. As in these protocols, the target for retroviral transduction will be CD34+ immunoselected hematopoietic cells. CD34+ cells have been shown to reconstitute patients undergoing ABMT (19-21). We have shown that these cells can be transduced with recombinant retroviral vectors in vitro (22), and preliminary data indicates that these retrovirally transduced cells contribute to engraftment in patients (C. Dunbar, unpublished observations, Appendix I).

An important difference between the neomycin marking protocols and the MDR-1 protocol is the use of a marker gene intended to change cell phenotype. While this will allow us to address new, biologically relevant questions, it also incorporates potential risks. It is theoretically possible that breast cancer cells may be transduced with the MDR-1 retroviral vector in this study. In order to reduce this risk, patients with evidence of bone disease evident on bone scan after induction therapy, or with histologic evidence of bone marrow involvement on bone marrow biopsy prior to or at harvest, will be excluded. Even in patients whose bone marrow is histologically negative for breast cancer, more sensitive immunohistochemical techniques show breast cancer cells in the marrow in a high proportion of patients with metastatic breast cancer (23). However, we believe that transduction of small numbers of breast cancer cells with the MDR-1 vector is very unlikely to adversely affect patient outcome. Breast cancer patients relapse primarily in sites of their original metastatic disease following ABMT, implying that contaminating tumor cells within unfractionated bone marrow do not significantly contribute to post-transplant relapse (2-4). Furthermore, we propose to use CD34 immunoselection to purify and transduce only a fraction of harvested bone marrow and mobilized peripheral blood cells. CD34 purification with the CellPro column will result in an additional 2-5 log reduction in any tumor cell contamination of harvested hematopoietic cells (Dr. E.J. Shpall, personal communication). Primary breast cancer cells grow poorly in culture (K. Cowan, unpublished observations), and it is not known whether remaining tumor cells would survive during the transduction period, or what proportion of surviving tumor cells would be transduced with the vector. Even in the unlikely event that viable, transduced tumor cells contributed to taxol and vinblastine resistant relapse, other treatment options remain. Antineoplastic drugs that are not substrates for Pgp, but which have activity in breast cancer, including antimetabolites (methotrexate, fluorouracil), and alkylating agents (thiotepa, cytoxan, melphalan, and cis-platinum), could be used for palliation. We plan to evaluate patients for evidence of relapse with transduced tumor cells. If patients relapse following transplant, biopsies of accessible tumor will be assayed for evidence of proviral integration, proviral mRNA expression, and immunologic evidence of Pgp expression.

3.0 Patient Eligibility

3.1 Histologically-documented Stage IV breast cancer.
3.2 Patients must have achieved at least a partial response to induction chemotherapy. Patients achieving a complete response are eligible for study.
3.3 Age: 18-60.
3.4 No history or clinical evidence of metastatic CNS disease.
3.5 Patients who have received treatment with more than two prior chemotherapy regimens or who have received mitomycin C are not eligible. Patients who have undergone a prior ABMT are excluded.
3.6 Normal cardiac function: No history of angina, myocardial infarction, CHF. LVEF must be >40%. Exercise treadmill test must be normal.
3.7 Creatinine clearance > 90cc/min, bilirubin <1.5., SGOT <2x normal, normal PT, PTT, calcium.
3.8 Negative HIV serology and hepatitis B surface antigen.

3.9 Adequate pulmonary function: DLCO >50% compensated for Hemoglobin; FEV >55%, pO_2 >60mm Hg.
3.10 Histologically negative bilateral bone marrow biopsies both prior to and at bone marrow harvest.
3.11 No evidence of metastatic disease to the bone by bone scan after induction chemotherapy. No prior pelvic radiation therapy and <20% of bone marrow previously radiated.
3.12 WBC >3500/mm^3 and platelets >100,000/mm^3.
3.13 ECOG performance status 0, 1, 2 and a life expectancy >60 days.
3.14 Negative beta-HCG serum pregnancy test in patients of child-bearing age.
3.15 Ability to give informed consent.
3.16 Good psychiatric and medical risk. All patients will undergo a psychosocial evaluation by the breast cancer psychiatric social worker.
3.17 Marrow harvest of >1 x 10^8 nucleated cells/Kg body weight (preprocessing) will be required for the patient to undergo transplantation with the MDR-1-transduced cells.

4.0 Pretreatment Evaluation

4.1 Complete history and physical examination with measurement of all palpable peripheral lymph nodes, liver, and other measurable lesions.
4.2 Hematological and Serological Studies: CBC with differential count, platelets, reticulocyte count, Westergren sedimentation rate, PT, PTT, Coomb's test, HIV serology, Hepatitis B surface Ag/Ab, CMV and HSV serology and HLA blood typing.
4.3 Chemistry Studies: Acute Care, Hepatic and Mineral panels, Uric Acid, LDH, CPK, Amylase, Thyroid function tests (Free T4, TSH), Total Protein, 24 hour urine for creatinine clearance and total protein, urinalysis, beta-HCG in patients of child-bearing age.
4.4 Radiological Studies:
4.4.1 Chest and abdominal CT scan.
4.4.2 Head CT scan.
4.4.3 Bone Survey and bone scan.
4.5 Pulmonary Function Tests with DLCO and arterial blood gas.
4.6 Nuclear Medicine cardiac ejection fraction (MUGA scan) and exercise treadmill test.
4.7 Bilateral iliac crest bone marrow biopsies and aspirates for routine staining.
4.8 CSF examination with cytology if disease clinically suspected.
4.9 Audiogram prior to and one month following discharge from hospital for bone marrow transplant.
4.10 Serum for baseline study for the presence of wild-type virus and antibodies to retroviral proteins.
4.11 Obtaining paraffin blocks of patient's breast cancer for immunohistochemistry for Pgp expression. Patients with accessible tumor will undergo fine needle aspirate and/or biopsy of their breast cancer for Pgp analysis of fresh tissue as well. Lesions to undergo FNA include breast, skin, nodal, lung or liver disease. Lesions to undergo biopsy include breast, skin, and nodal disease.
4.12 1 purple top tube of blood and a marrow aspirate will be obtained for DNA and RNA analysis as controls.

5.0 Treatment Plan

5.1 Patient Registration: All patients must be registered on study by the clinical associate by contacting Orkand at 402-1732 (8:30 A.M. - 5:00 P.M., Monday - Friday) with the appropriate patient information. Patients must also be officially taken off study by contacting Orkand Personnel.

5.2 All patients will have a double lumen apheresis Hickman placed prior to PBSC harvest. This will be used during the administration of high dose chemotherapy, PBSC and marrow reinfusion and during the period of myelosuppression.

5.3 Patients with metastatic breast cancer will be treated with 4-5 cycles of induction chemotherapy on an NCI Medicine Branch Treatment protocol with either FLAC (5-fluorouracil, leucovorin, doxorubicin, cyclophosphamide) with GM-CSF versus pIXY321 or with taxol/cyclophosphamide and G-CSF. Patients achieving at least a partial response with induction chemotherapy will be eligible for transplant. Patients who have not achieved a PR but who are responding after 4-5 cycles may be treated with additional cycles in an attempt to achieve a PR. Such patients will be eligible for transplant if a PR is obtained. Patients who have achieved a PR after 4-5 cycles and who continue to respond may be treated with additional cycles until a CR or best response is achieved. Patients who achieve an early CR will also receive 4-5 cycles of induction chemotherapy. A maximum of 9 induction cycles will be administered.

5.4 After bone marrow recovery from induction chemotherapy has occurred (ANC >2000/mm^3 and platelets >100,000/mm^3), patients will undergo first bone marrow harvest and then PBSC harvest. At least two weeks will elapse from recovery of the blood counts after induction therapy to bone marrow harvest.

5.4.1 PBSC Mobilization and Harvest - see Section 6.1 for details of apheresis procedure. Throughout, patients will receive standard antiemetic regimens and intravenous hydration to maintain urine output >100cc/hr and to normalize electrolytes. PBSC mobilization and harvest will be conducted on the inpatient 13E or 12W oncology units and in the NIH Transfusion Medicine Department.

Day 1: Begin intravenous hydration 3 liters/M^2/day normal saline. Continue x 24 hours. If urine output is less than 100cc/hr, consider furosemide.

At least 4 hours after beginning hydration, cyclophosphamide 4 g/M^2 will be diluted to a final volume of 500mL with NS and infused I.V. over 2 hours.

MESNA 800mg/M^2 I.V. over 30 minutes at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 hours post-cyclophosphamide. In addition, 800mg/M^2 Mesna will be added to the cyclophosphamide and will be infused over the 2 hours.

Day 2: Begin G-CSF at a dose of 10 µg/kg/day subcutaneously.

When the total white blood cell count recovers to >2000/mm^3 begin daily leukapheresis, continued until at least 3 x 10^8 nucleated cells/kg are collected. G-CSF will be discontinued after the final leukapheresis.

5.4.2 At least 4 weeks will elapse from the time of WBC and platelet recovery following PBSC harvest to bone marrow harvest. For bone marrow harvest to take place, patients' ANC must be >1500/mm^3 and platelets >100,000/mm^3. Bone marrow will then be harvested as in Appendix II.

5.5 **ICE Chemotherapy Regimen**

5.5.1 Drug Schedule

Drugs:	Days	1	2	3	4	5
Ifosfamide 4 g/M^2/day I.V. q.d. days 1 to 4 (4 days)		x	x	x	x	
CBDCA 600 mg/M^2/day CIV days 1 to 4 (72 hours)		xxxxxxxxxxxxxxxxxx				
Etoposide 250 mg/M^2/q12 hrs I.V. on days 1 to 3 (6 doses)		xx	xx	xx		

MESNA	x	x	x	x
6400 mg/M^2/day I.V.				
on days 1 to 4 (4 days)				
As outlined below:				
MESNA loading dose:	800 mg/M^2 I.V. with Ifosfamide (hr 2-4)			
Constant infusion:	800 mg/M^2 I.V. over 3 hr (hr 4-7)			
Bolus doses:	800 mg/M^2/dose I.V. q 3 hr x 6 (hr 7,10,..)			

Bone Marrow and PBSC Reinfusion Day 7

Day 7: Begin G-CSF 10μg/kg SC daily until ANC >2000/mm^3 on 3 successive days.

5.5.2 ICE Administration: On admission and for 3 months following discharge, patients must take Bactrim DS 1 po BID Q Monday/Tuesday/Wednesday. All blood products, excluding transplanted cells, must be irradiated and a double luman Hickman catheter must be in place. MOUTH CARE: Peridex mouthwash 30 cc po swish and spit q8h; NaH_2CO_3 Rinse (2 tablespoon/500 cc sterile H_2O) 120 cc po swish and spit q4h; Clotrimazole Troche 10 mg suck q4h and if not tolerated, Nystatin 20 cc po swish and swallow q4h. ANTI-EMETIC REGIMEN (suggested): Ondansetron 0.15 mg/Kg IV q4 or q6 hours; Ativan 1-2 mg I.V. q8-12. Do not over-sedate patients and DO NOT GIVE STEROIDS. Droperidol and diphenhydramine are recommended for breakthrough nausea and vomitting.

Hydration will start 24 hour prior to ICE and will be D5NS at 300 ml/hr on day 0 and repeat creatinine clearance will be obtained. Chemotherapy should start at 11:00 A.M. on day 1. Hydration will continue for at least 7 days after completion of ifosfamide if tolerated. Hydration will not be stopped without discussion with the principal investigator unless necessary for medical indications, e.g., CHF. Furosemide may be administered as needed to maintain urine output at >150 cc/hour and to reduce patient weight gain. Please note that many patients will initially gain 5% fluid body weight followed by a spontaneous diuresis. During ifosfamide administration (days 1-4), urine should be tested for hemoglobin daily and if gross hematuria develops, the principal investigator should be notified immediately and the next dose of ifosfamide held. If gross hematuria develops, a three-way Foley catheter is to be inserted and continuous bladder irrigation with 1 L/hr normal saline continued 24 hours beyond completion of chemotherapy (day 4).

All patients will develop a metabolic acidosis (decreased serum CO_2) because of a transient proximal renal tubular acidosis (H_2CO_3 loss) and accumulation of the ifosfamide by-product, chloracidaldehyde. The serum CO_2 level generally begins to fall by the third day of chemotherapy and reaches a nadir 3 to 4 days later. When the serum CO_2 level falls to 18, the I.V. fluid changes to D5 1/2 NS + 50 meq/L Na H_2CO_3. If the CO_2 continues to fall to < 16, 12 hours later, the I.V. fluid should be changed to D5 1/4 NS + 100 meq/L Na H_2CO_3. The Na_2CO_3 should be removed with the CO_2 is stable and >22.

For a serum potassium < 3.5 mmol/L administer 15 mEq of potassium I.V. over 1 hour. Recheck serum potassium 1 hour after completing the potassium infusion. Alternatively potassium may be administered in hydration fluids. Patient's clinical associate should be called for a potassium <3.5mmol/L.

Ifosfamide, admixed with 20% MESNA on a weight basis, will be administered in 100 ml D5W intravenously over 2 hours, daily x 4 days,

immediately following etoposide. MESNA will be administered first as a loading dose admixed with ifosfamide over 2 hours (hr 2-4, followed immediately by a 3 hour continuous infusion, hr 4-7). At completion of the infusion, MESNA is administered every 3 hours as an intravenous injection over 15 minutes x 6 doses (hr 7, 10, 13, 16, 19, 22) x 4 days.

Etoposide will be mixed in 500 ml NS and infused over 90-120 minutes (maximum concentration of 1 mg/ml). Etoposide will be started prior to the ifosfamide on the first day of ICE and continued every 12 hours for 6 doses, ending on the third day of ICE.

CBDCA will be administered as a continuous infusion over 72 hours starting on the first day of ICE. A 24-hour supply will be placed in 500 ml of D5W and infused through the lumen not being used for the ifosfamide/mesna.

Management of fever and neutropenia should be done in collaboration with the Infectious Disease team and the principal investigator. Patients should not be discharged until afebrile and an absolute granulocyte count >500/mm^3 x 2 consecutive days has been achieved. Patients may be discharged while receiving G-CSF. Patients may be discharged while still requiring platelet support provided there is no clinical bleeding and arrangements are made for outpatient platelet support.

5.6 After ICE chemotherapy on days 1-4, patients will undergo bone marrow and PBSC reinfusion on Day 7. The bone marrow and PBSC to be reinfused will consist of the CD34+ stem cells that have been transduced with a retroviral vector bearing the MDR-1 gene as well as the 70% of the bone marrow harvest and PBSC that were cryopreserved without transduction.

5.7 Beginning on Day 7 patients will be treated with G-CSF 10μg/Kg SC daily until the ANC >2000/mm^3 on three successive days.

5.8 Patients will be monitored with daily CBC, platelet count, differential, electrolytes, BUN, creatinine, calcium, phosphate, albumin, magnesium, and liver function tests.

5.9 Patients with residual metastatic disease or progressive disease after bone marrow and PBSC transplant will be offered treatment with taxol chemotherapy provided their ANC >1500/mm^3 and plts >90,000/mm^3. SGOT, SGPT and total bilirubin must be <2x normal unless there is evidence of tumor involvement in which case up to 4x normal SGOT and SGPT are allowed and a total bilirubin up to 1.8mg/dl. Patients' performance status must be ECOG 0, 1, 2. Patients must have recovered from the toxicities of bone marrow transplantation. Taxol or vinblastine therapy may begin after the restaging studies have been obtained one month after their discharge from the hospital. Patients' disease sites will be imaged after every 2 cycles of taxol therapy. Patients will continue on taxol as long as their disease is stable or responding. Taxol will be administered by I.V. infusion at 175mg/M^2 for 3 hours by a central venous catheter. Dexamethasone, diphenhydramine and cimetidine premedications will be given in standard doses. Treatment with taxol will be repeated every 21 days. Patients previously treated for metastatic disease with taxol will be treated with vinblastine 1.5mg/M^2/day I.V. bolus for 5 days. The same eligibility criteria as for taxol administration will apply. G-CSF 5μg/Kg will be given SC daily starting the next day and at least 24 hours after the end of taxol or vinblastine chemotherapy. G-CSF will be continued until the ANC >10,000/mm^3 on two successive determinations. Vinblastine treatment will be repeated every 21 days. A minimum of 24 hours must elapse between the last dose of G-CSF and the next cycle of taxol or vinblastine.

5.10 Doses of taxol and vinblastine on successive cycles will be escalated by 10% to achieve a nadir ANC count <1000/mm^3. Once this is achieved, the dose of the drug

will be held constant unless dose modification is needed for toxicity (Section 7.0). A CBC with differential will be obtained two times per week while receiving taxol or vinblastine therapy.

5.11 Patients will be treated with taxol or vinblastine and G-CSF until stable disease has been documented over 3 cycles or until progressive disease.

6.0 Cell Harvesting, Processing, Transduction, and Reinfusion

6.1 Peripheral Blood Stem Cell Collection

6.1.1 Patients will undergo daily apheresis on a continuous flow apheresis instrument such as a Spectra 2991 (COBE) or a Fenwal CS3000 (Baxter) following cytoxan/G-CSF administration beginning on the day when the total leukocyte count reaches 1000/mm^3. The first day's collection will be processed and cryopreserved as per the original protocols. The collection on the day after the collection is first $\geq 1 \times 10^8$/kg nucleated cells/kg will be CD34-enriched and transduced with the MDR-1 vector. No further processing of the collected cells is performed prior to the CD34-enrichment procedure (Section 6.3).

6.1.2 Collections on subsequent days will proceed as necessary until $\geq 3 \times 10^8$ total nucleated cells/kg are collected. These collections will be processed and cryopreserved directly without CD34-enrichment or transduction.

6.1.3 If no collection is $\geq 1 \times 10^8$ nucleated cells/kg, then the patient will not have peripheral blood cells CD34-enriched or genetically modified.

6.2 Bone Marrow Harvest

6.2.1 The following preoperative tests must be obtained prior to harvest: CBC, platelets, PT, PTT, electrolytes, BUN, Cr, glucose, calcium, phosphate, albumin, magnesium, liver function tests, urinalysis, Chest X-ray, ECG. Type and Cross 3 units irradiated PRBC for the OR.

6.2.2 Patients' bone marrow will be harvested after 4-5 cycles of induction chemotherapy and after the PBSC harvest has been done as below. Bone marrow harvest is arranged by contacting Dr. Wyndham Wilson (496-6404 or page 104-2399-7) or George Bryant (496-9521 or page 104-2556-7) at least 1 week before the procedure. Patients must have an AGC>1500/mm^3 and platelets >100,000/mm^3. Marrow collection is performed in the Clinical Center operating rooms under general anesthesia. The patient must be admitted to the hospital by 2:00 P.M. on the day prior to marrow collection and evaluated by the anesthesiologist and BMT team.

6.2.3 The entire marrow is processed on a cell processing instrument such as the Fenwal CS3000 or the Spectra 2991 to obtain a buffy coat preparation. ABMT requires a minimum of 5×10^7 mononuclear cells/kg body weight. In general, $>1 \times 10^8$ nucleated cells/kg body weight are harvested. Every attempt will be made to harvest $>3 \times 10^8$ nucleated cells/Kg body weight before processing. If it is possible to obtain $>1 \times 10^8$ nucleated cells/Kg before albumin processing, 70% of the purified marrow will be immediately cryopreserved using standard techniques. The remaining 30% will be used for retroviral transfection. If $<1 \times 10^8$ nucleated cells/Kg body weight are harvested, the entire purified marrow will be frozen immediately. In this case, no bone marrow cells will be used for retroviral transduction.

6.3 Antibody Treatment and Wash

6.3.1 The primary buffy coat bone marrow or apheresis product is transferred to a 600 ml bag for antibody incubation. One 3.0 mg vial of CellPro 12.8 biotinylated murine IgM anti-human CD34 antibody is thawed at room temperature for 20 minutes.

6.3.2 RPMI media and 1.5 ml of processed autologous plasma are added to bring the total volume in the bag to 150 ml. The entire 3.0 mg vial of antibody is added to the bag. The cells are incubated at **room temperature** for 25 minutes.

6.3.3 The cells are washed with one liter of PBS and brought back to a total volume of 300 ml with PBS. Samples are removed for counting and analysis.

6.4 CEPRATE Column Set Up and Run

6.4.1 During the antibody incubation the CEPRATE™ SC Stem Cell Concentrator is set up and primed.

6.4.2 Transfer packs, Reagent bags and Collection bags are prepared and labelled per manufacturers instructions. 1% autologous plasma is used for the PBS/Protein bag.

Reagent Bags:	PBS	300 ml PBS
	PBS/PROTEIN	200 ml PBS/2ml plasma
	PBS	1 liter
Collection Bags:	STEM CELL BAG	5 ml plasma in 150ml bag
	WASH	empty 300 ml bag
	UNABSORBED	empty 1 liter bag
	PRIME	empty 600 ml bag

6.4.3 The CEPRATE instrument is turned on and the computer performs the electrical check. The sterile tubing sets, prefilter, precolumn and column are installed via the LOAD REAGENT, SET UP, and RUN menus. The PRIME menu is then run to allow the instrument to automatically remove any air from the system.

6.4.4 The bag containing the antibody-treated cells is installed on the CEPRATE instrument. The RUN PROCESS menu is run and the cells flow through the filter and columns automatically. The CD34+ antibody coated cells bind to the avidin conjugated beads in the column, while the unlabelled cells pass through into the "unabsorbed" bag. The bound cells are then removed from the column by gentle agitation and collected automatically in the "stem cell" bag. The entire procedure should be monitored using the System Functions Table.

6.4.5 At the end of the run, instructions on the screen are followed to remove the "stem cell", "wash" and "unabsorbed" bags from the machine.

6.5 Volume Reduction

6.5.1 The stem cell bag will contain an approximate volume of 100 ml. Heparin will be added to a final concentration of 10 units/ml.

6.5.2 The stem cell bag contents will be transferred to 50 cc conical tubes coated with a small volume of autologous plasma, and the bag will be rinsed with heparinized (10 units/ml) TC199 media. Aliquots will be removed for counting and analysis.

6.5.3 The tubes will be centrifuged at 1200 rpm (420 g) for 5-10 minutes.

6.6 Transduction of the CD34-Enriched Bone Marrow and Peripheral Blood Stem Cells

6.6.1 Clinical-grade, fully certified viral supernatant obtained from Genetic Therapy, Inc. will be used to transduce CD34 immunoselected cells from bone marrow and peripheral blood. These clinical grade supernatants, derived from the G1MD-C5 producer clone, will contain between 2 and 20 x 10^5 infectious viral particles per ml.

6.6.2 The final concentration of CD34-enriched cells at the initiation of transduction will be 1 X 10^5/ml. Sufficient supernatant for the transduction will be thawed at 37 degrees in a water bath. Preservative free protamine sulfate (i.v. grade, Elkins-Sinn Inc., 10,000 ug/ml) will be added to the supernatant to reach a final concentration of 4 ug/ml. Human recombinant interleukin 3 will be added to the supernatant to reach a final concentration of 20 ng/ml. Human recombinant stem cell factor will be added to the supernatant to reach a final

concentration of of 100 ng/ml. Human recombinant interleukin 6 will be added to the supernatant to reach a final concentration of 50ng/ml.

6.6.3 The CD34-enriched bone marrow or peripheral blood cells will be resuspended in this transduction media at a concentration of 1 X 10^6 cells/ml. The suspension will be transferred to Nunc T175 flasks and transduction media added to bring the final concentration of the cells to 1 X 10^5/ml.

6.6.4 The flasks will be incubated at 37 degrees C in 5% CO_2 and 80% humidity for 20-24 hours.

6.6.5 The cells and media will be removed from the flasks and pelleted in 50 ml conical tubes at 1200 rpm for 5-10 minutes. The pellets will be resuspended the same volume of fresh transduction media and transferred back into the same T175 flasks.

6.6.6 After another 24 hours incubation the cells will again be removed from the flasks, pelleted, resuspended in fresh transduction media and returned to the flasks.

6.6.7 After a third 24 hour incubation, the cells will be removed from the flasks and transferred into 50 ml conical tubes. The flasks will be rinsed once with 20 ml PBS each. 5 ml of trypsin (Gibco) will be added to each flask and allowed to sit at room temperature for 5 minutes to dislodge cells adherant to the flask. The flasks will be rinsed again with 20 ml TC199.

6.6.8 The cells will be pelleted at 1200 rpm for 5-10 minutes and washed twice more with heparinized TC199. Aliquots of cells will be removed for counting, microbial cultures, and other analysis (Section 6.6.9). If no bacterial or fungal contamination is found on gram stain and cultures, the thawed, transduced cells will be admixed with the thawed, non-transduced bone marrow and peripheral blood cells and reinfused into the patient on Day 7 of treatment (see Section 6.8).

6.6.9 After the infection protocol is completed, the CD34+ cells will be evaluated for the efficacy of transduction. DNA will be extracted from an aliquot of cells and analyzed by PCR for proviral MDR-1 cDNA. Additional aliquots will be analyzed by a Rhodamine efflux assay and by immunofluorescence using the MRK-16 antibody for MDR-1 gene expression. The clonogenic plating efficiency in methylcellulose cultures will be determined as a measure of enrichment and cell viability.

6.7 Cryopreservation of Transduced CD34-Enriched Cells

6.7.1 Cryopreservation solution will be prepared in a 50 ml conical tube and kept at 4°C: 9 ml heparinized TC199 media + 3 ml DMSO

6.7.2 The supernatants will be removed from the pelleted cells and the pellets will be pooled in a volume of less than 2.25 ml, and a cell count will be performed. If the total cell number is < 100 X 10^6, the cells will be brought to a total volume of 2.25 ml with heparinized TC199. If the total cell number is $\geq$ 100 X 10^6, the cells will be brought to a volume of 4.5 ml with heparinized TC199.

6.7.3 2.25 ml of cells will be placed into 1 (or 2) labelled 5 ml freezing tubes. 8 ml of autologous plasma is added to the cryopreservation solution, and then 2.25 ml of this mixture is added to each freezing tube. The samples will then be frozen in a controlled rate freezer and stored in vapor phase of liquid nitrogen.

6.8 Reinfusion of Non-transduced and Transduced Cells

6.8.1 Infusion Instructions: The bone marrow and PBSC will be infused 3 days following the last ifosfamide dose by the transplant team. Non-transduced and transduced cells will be infused simultaneously. Premedicate patients with hydrocortisone 100mg IVP, acetominophen 650mg PO and diphenhydramine 25 mg IVP 30 mins before reinfusion. Cells are infused intravenously at the bedside over 30 - 120 minutes without a filter after rapid thawing in a 37

degree water bath. Total volume is expected to be 100-500 cc. Possible toxicities include volume overload, which can be treated by slowing the infusion and using diuretics, and transient dyspnea due to mast cell degranulation induced by DMSO, which can be treated with additional antihistamines and steroids. All patients will have their vital signs monitored q15 minutes x 4 and q30 minutes x 2. Additional demerol and benadryl are to be used as needed. Hypotension is to be corrected with fluid and pulmonary symptoms with 2L/min supplemental oxygen.

6.8.2 Infusion of Transduced CD34-Enriched Cells

a) The cell vials will be removed from liquid nitrogen and thawed at 37°C in a water bath within a ziplock bag.

b) Thawed cells will be transferred to a 50 cc conical tube in a laminar flow hood. 5 ml of heparinized TC199 will used to rinse each freeze tube and then will be added dropwise to the cells. Over a period of five minutes the cells will be diluted to a total volume of 30 ml with heparinized TC199.

c) The cells will be drawn into a labelled 60cc syringe and a sample will be removed for counts and analyses. The stem cell concentrate will then be infused into the patient over 5 minutes. The above procedure will be repeated for each vial of transduced bone marrow or peripheral blood CD34-enriched cells.

7.0 Dose Modifications

Taxol or Vinblastine: Cycles will be delayed one week for an ANC <1500/mm^3 or platelets <90,000/mm^3 on the day that chemotherapy is due. The taxol or vinblastine dose will be escalated by 10% each cycle to achieve a nadir ANC <1000/mm^3. The dose of taxol or vinblastine will be decreased by 10% for an ANC <500/mm^3 for >5 days or platelets <20,000/mm^3 during previous cycle.

7.1 If after a one week delay, the ANC is <1500/mm^3 or platelets <90,000/mm^3, treatment with taxol or vinblastine can be given as below:

If ANC is:	Taxol or Vinblastine Dose:
1200-1499	75 %
900-1199	50 %
less than 900	no drug
If platelet count is:	**Taxol or Vinblastine Dose:**
75,000 - 89,000	50 %
<75,000	no drug

Non-Hematologic Toxicity:

Grade 1 or 2: No dose reductions.

Grade 3 or 4: Hold therapy until toxicity resolves and then give next cycle with a 25% dose reduction.

Recurrent Grade 3 or 4 toxicity: Off-Study

7.2 G-CSF Dose Modification: For Grade 3 toxicities that are, in the judgement of the Principal Investigator, due to G-CSF, the dose of G-CSF should be reduced to 50% of the original dose. For Grade 4 toxicities that are, in the judgement of the Principal Investigator related to G-CSF, the G-CSF should be stopped and not resumed. For toxicities of Grade 2 or less that are considered by the Principal Investigator to be related to G-CSF, an effort should be made to ameliorate the toxicity through use of acetaminophen and/or diphenhydramine. If a patient cannot tolerate a dose of 5.0 µg/kg G-CSF per day during BMT or 2.5µg/Kg during vinblastine treatment, the G-CSF will be stopped.

8.0 On Study Evaluation

8.1 Daily CBC, platelet count, differential, electrolytes, glucose, BUN, creatinine while hospitalized and 2 times per week until day 60 post-transplant for patients not receiving taxol or vinblastine. Patients treated with post-transplant taxol or vinblastine will have twice weekly CBC, platelet count, and differential performed.

8.2 Three times per week calcium phosphate, magnesium, albumin, total protein, SGOT, SGPT, alkaline phosphatase, LDH, total bilirubin, PT, PTT, fibrinogen, TT while hospitalized then weekly until day 60 post-transplant. For patients receiving taxol or vinblastine, electrolytes, calcium, phosphate, albumin, BUN, creatinine, glucose and liver function tests will be obtained every 3 weeks.

8.3 Weekly chest x-ray, urinalysis until hospital discharge.

8.4 One purple top and one green top tube will be obtained two times a week while patients are hospitalized to study hematopoietic cells for the presence of the MDR-1 provirus by PCR, and Pgp expression and function by immunohistochemistry and the rhodamine efflux assay.

8.5 Bone marrow aspiration and biopsy will be obtained on Days 14, 28, and 42 post-transplant for PCR, clonogenic assays and FACS analysis of hematopoietic cells in all patients.

8.6 Patients will undergo a restaging evaluation of their known sites of metastatic breast cancer one month after their discharge from the hospital. Patients with residual or progressive disease will be eligible for treatment with vinblastine or taxol after this restaging evaluation.

8.7 For patients receiving taxol or vinblastine, blood samples will be obtained on three successive days prior to beginning chemotherapy and at the end of each cycle of therapy to study the hematopoietic cells for the presence of the MDR-1 provirus by quantitative PCR and to study Pgp expression by immunohistochemistry. Bone marrow aspirates and biopsies will be obtained after marrow recovery from cycles 1 and 3 of taxol or vinblastine to study the effects of chemotherapy on proviral copy number. We will also study expression of the vector derived MDR-1 mRNA in bone marrow cells by RT-PCR, immunohistochemistry, FACS, and *in situ* hybridization techniques.

8.8 Evaluation after Day 60 post-transplantation: Patients not receiving taxol or vinblastine therapy will return to NCI monthly. Monthly evaluation will include CBC, platelets, differential, electrolytes, liver function tests, BUN, creatinine, calcium, albumin, magnesium. Peripheral blood samples for PCR analysis will also be obtained (1 green and 1 purple top tube).

8.9 Bone marrow aspirates will be obtained every three months in patients who received transduced marrow and who are receiving treatment with taxol or vinblastine. These samples will be analyzed for the MDR-1 provirus by PCR. FACS analysis and clonogenic assays with and without taxol will also be performed. PCR will be performed on the hematopoietic clones that survive incubation in taxol.

8.10 Patients will undergo restaging of their metastatic breast cancer every 3 months. This will include a CXR every 3 months and CT and bone scans of sites of previous disease.

8.11 A creatinine clearance will be obtained prior to hospital discharge.

8.12 An audiogram will be obtained prior to and one month after ABMT.

8.13 Biopsies will be done of accessible tumor (skin, lymph nodes, pleural effusions, bone marrow, or liver or lung disease accessible by CT - guided FNA) at the time of relapse to assay for the presence of the MDR-1 gene by PCR.

8.14 Apheresis following taxol or vinblastine/G-CSF (during the nadir recovery period) will be performed on patients with circulating cells or bone marrow cells that contain the transferred MDR-1 cDNA. For patients in a clinical CR or not otherwise receiving taxol or vinblastine/G-CSF, and whose marrow or peripheral cells contain the MDR-1

provirus, apheresis will be performed to study the MDR-1 transduced populations. Flow cytometry studies using the MRK-16 antibody and/or rhodamine efflux will be done on cells collected by apheresis to study Pgp expression and function. Furthermore, these cells will be sorted by FACS and the various subpopulations tested for the MDR-1 provirus by PCR and *in situ* hybridization. Hematopoietic clonogenic assays will be grown with and without the addition of taxol.

8.15 Serum for wild type virus and Western blot analysis will be obtained every 3 months.

8.16 Upon the death of a patient, an autopsy will be requested and multiple organ sites will be analyzed by PCR for the presence of the MDR-1 gene.

9.0 Statistical Considerations

The main objectives of this pilot trial are to study the feasibility of obtaining bone marrow engraftment with hematopoietic stem cells bearing the MDR-1 provirus after high dose chemotherapy and to study whether the transduced cells can be positively selected by post-ABMT chemotherapy. The trafficking and time course patterns of the MDR-1 marked cells in the peripheral blood and bone marrow will be studied by serial sampling of these sites during and after bone marrow engraftment. Patients with residual or progressive breast cancer post-ABMT will be treated with taxol or vinblastine therapy plus G-CSF. The dose of taxol or vinblastine will be increased by 10% each cycle to achieve a neutrophil nadir $<1000/mm^3$ (Section 5.10). When this nadir count has been achieved, the dose of taxol or vinblastine will be held constant for successive cycles unless dose reductions for toxicity are needed (Section 7.0). Blood and bone marrow samples will be obtained to determine if positive selection for the MDR-1 transduced hematopoietic cells has occurred with taxol or vinblastine therapy. Three blood samples obtained before and after chemotherapy will be analyzed in triplicate by quantitative PCR for the percentage of cells containing proviral MDR-1. (The PCR methodology and the procedure for determining copy number are outlined in Appendix I.) The null hypothesis for this pilot, feasibility study is that taxol or vinblastine therapy will not increase the number of circulating and bone marrow hematopoietic cells that express the transduced MDR-1 gene. Each patient will act as her own control with regard to the percentage of cells carrying proviral MDR-1 pre- and post-chemotherapy.

The table below shows the statistical power of the assay. Although our assay has a measured standard deviation of 0.28 of the mean (Appendix I), the table below assumes a value of 0.3. The first column is the factor by which the treatment actually increases the percentage of circulating MDR-1-transduced cells, and the second column is the probability of finding a significant difference. In an individual patient, if any single cycle of taxol (vinblastine) treatment resulted in a three fold increase in proviral copy number, our assay would detect a difference significant at the two-tailed p=0.05 level in 92 percent of the cycles. We anticipate treating 18 patients, and each patient with residual disease or relapse will likely receive several cycles of salvage chemotherapy.

Factor	Power (SD=0.3)
2.0	57.0%
2.5	80.0%
3.0	92.0%
3.5	97.0%
4.0	99.0%
4.5	99.7%
5.0	99.9%

We will calculate the mean of nine measurements of proviral copy number both before and after each cycle of chemotherapy. Amplification of transduced cells will be defined as an increase in the mean copy number at a two tailed value of p=0.05 or less.

Although the primary endpoint of this trial will be the percentage of blood cells that contain the MDR-1 provirus post-transplant and following taxol therapy, we will review the severity and duration of myelosuppression with each cycle of chemotherapy. The nadir counts for each patient will be correlated with changes in the mean proviral copy number. A beneficial effect of MDR-1 gene transfer in ameliorating myelosuppression would be expected only if a substantial amplification of the transduced cell population occurs with repeated cycles of taxol or vinblastine.

Ten to twelve patients per year will be treated on this pilot trial. A maximum of 18 patients will be treated. Consideration will be given to treating fewer than 18 patients if no gene marking of the marrow or peripheral blood occurs in the first 6-8 patients treated with post-ABMT taxol or vinblastine. This study will be stopped if the first 2 patients fail to engraft or if 2 patients fail to engraft during the duration of the trial.

10.0 Off-Study Criteria

10.1 Progressive disease while receiving taxol or vinblastine therapy post-ABMT.
10.2 Recurrent grade 3 or 4 toxicity with taxol or vinblastine therapy post-ABMT.
10.3 Patients may be taken off-study by the Principal Investigator if it is deemed medically inappropriate to continue with therapy.
10.4 Patients may choose to withdraw from study at any time.

11.0 Reporting of Data and Adverse Drug Reactions

This study will be CTMS monitored. Data will be submitted to CTMS at least every two weeks. The NCI/DCT Case Report or ACES will be used to report to CTMS.

Reporting of adverse drug reactions (ADR) will be made using the Division of Cancer Treatment Common Toxicity Criteria and Toxicity Criteria for ABMT Studies Supplementary Toxicity Criteria (Appendix V) for reference according to the guidelines published by the DCT, NCI. The ABMT Toxicity Criteria will be the primary guideline with the Common Toxicity Criteria used as a supplement. Report by telephone to IDB within 24 hours (301-230-2330, available 24 hours per day) the toxicities below. A written report should follow within ten working days to: Investigation Drug Branch, P.O. Box 30012, Bethesda, Maryland 20824. Adverse Drug Reactions must also be reported to the Chairman, ICRS at the same time as IDB. Reporting guidelines are summarized as follows:

All life-threatening events (Grade 4, except for Grade 4 myelosuppression) which may be due to administration of investigations drug(s).

All fatal events (Grade 5).

All first occurrences of any previously unknown toxicity (regardless of grade).

12.0 Response Criteria

Complete Response: No evidence of disease on restaging one month after bone marrow transplant persisting for 4 weeks.
Partial Response: A 50% or greater reduction in the size of measurable lesions as defined by the sum of the products of the longest perpendicular diameters of all measurable indicator lesions for at least one month.
Minor Response: A 25% to 49% reduction in the size of the measurable lesions as defined by the sum of the products of the longest perpendicular diameters of all measurable indicator lesions for at least one month.

Stable Disease: Tumor size not showing evidence of response or progression.
Progressive Disease: An increase of 25% or more in the size of measurable lesions as defined by the sum of the products of the longest perpendicular diameters of all measurable indicator lesions compared to the smallest previous measurement, or the appearance of a new lesion.

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INSTITUTE: NATIONAL CANCER INSTITUTE

STUDY NUMBER:___ PRINCIPAL INVESTIGATOR:Joyce O'Shaughnessy, M.D.

STUDY TITLE:Retroviral Mediated Transfer of the Human Multidrug Resistance Gene (MDR-1) into Hematopoietic Stem Cells during Autologous Transplantation after Intensive Chemotherapy for Metastatic Breast Cancer

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all that take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in this study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you may have about this study with the staff members who explain it to you.

NATURE OF STUDY

Autologous bone marrow transplantation, the return of one's own bone marrow following high dose chemotherapy, is a treatment with potential benefit for metastatic breast cancer. Autologous bone marrow transplantation and its risks have been explained to you in a separate Informed Consent document and you have agreed to be enrolled in our transplantation protocol. You are now also being asked to take part in a research study in which a portion of the bone marrow and circulating blood cells will be marked with a special gene before the cells are put back into you.

Genes are the hereditary material present in all body cells. They control the proteins made in the cell, which in turn determine how the cell functions. When special genes are inserted into cells, the cells are referred to as "marked" since they can be distinguished in the laboratory from identical cells without the insertion. Putting a gene into yourells is an experimental approach we are studying to try and improve future therapies.

In this protocol we will treat you with high dose chemotherapy and then return your bone marrow and circulating blood cells, some of which have been "marked" with the special gene described below. The goal is to determine whether it is possible to put a new gene into bone marrow cells, genes that will stay in your body for several months or more. If this could be accomplished, it would represent an important potential advance in the treatment of many diseases. We may also gain information that could improve the safety and usefulness of autologous transplantation in breast cancer. We will also study whether the added gene will increase in numbers after

administration of chemotherapy should you need further treatment after bone marrow transplantation.

We will use a new technology, "retroviral mediated gene transfer," in an attempt to put a human gene called MDR-1 into some of your bone marrow and circulating blood cells before they are transplanted back into your body. Then we will test bone marrow and circulating blood cells periodically to see whether the MDR-1 gene is in the cells and how long the gene stays there. The MDR-1 (multidrug resistance) gene helps cells to become resistant to certain chemotherapy drugs including doxorubicin, taxol, and vinblastine. We hope that a portion of your bone marrow and peripheral blood cells will contain the MDR-1 gene after your transplant. Should your breast cancer recur, we will offer you treatment with the chemotherapy drugs taxol or vinblastine. The ultimate hope is that the MDR-1 gene will protect your bone marrow from the toxic effects of chemotherapy although this may not occur during these early stages of development of this treatment approach. It is important to understand that the introduction of this gene may not benefit you in any way. It is possible that this study may lead to improved bone marrow transplantation therapies for others in the future. It is possible that the MDR-1 gene may protect a portion of your bone marrow from the toxic effects of future chemotherapy, but we do not know whether this will occur at this time.

EVALUATION AND PROCEDURES

The following procedure will be used to insert the gene into your bone marrow and blood cells. Your bone marrow cells will be obtained by bone marrow harvest in the operating room using the regular procedure for harvest. It will not be necessary to take any more bone marrow than is normally taken. Your circulating stem cells will be obtained by removing blood through a vein, separating the immature "stem" cells of interest, and returning the remaining blood to you through a vein. No more than 30% of marrow and blood stem cells will undergo retroviral-mediated gene transfer and the rest will be handled in the usual fashion. In this 30% portion, the cells to undergo the gene transfer will be separated from the other bone marrow and blood cells by attaching a special antibody to them and then physically removing only the cells that can pick up the antibody. This procedure is called "immunoadsorption" and it allows us to separate the most immature stem cells which will be the ones used for the gene transfer. The laboratory machine (called an immunoadsoprtion column) used in this protocol is experimental. Once the immature blood cells have been separated, the antibody will be removed. These cells will then be mixed together with two substances

called growth factors that help the blood cells divide and with a retroviral vector that carries the MDR-1 gene. Vectors are organisms that carry material from one cell to another. Our vector is a retrovirus found in mice which has been 'disabled' to make the virus incapable of replicating. The vector enters the blood cells and puts the MDR-1 gene into the genetic material called chromosomes. Once in the chromosomes, the MDR-1 gene will survive as long as the cell survives. The MDR-1 gene makes the blood cells resistant to drugs and "marks" them so we can identify them. The blood cells, vector and three growth factors are put together in a mixture called a culture. After 3-6 days, the cells are removed from the culture and "washed" to remove any extra virus or growth factor attached to them. The "marked" cells are then frozen and are returned to you at the time of transplantation along with the 70% of your bone marrow and blood cells that have not been exposed to the vector. The untreated cells should be enough to produce blood count recovery in the expected time period after high dose chemotherapy.

The standard blood drawings and tests that are required following bone marrow transplantation will be performed. These have all been explained to you in a separate consent document. During your hospitalization and after you are discharged, blood samples will be drawn to study your blood counts and to determine whether the MDR-1 gene is present in the blood cells. Approximately two extra teaspoons of blood will be drawn three times a week for these research studies. Bone marrow aspirations will be obtained at 2, 4, and 6 weeks after your bone marrow is given back to you to test for the presence of the MDR-1 gene. You will be given local anesthesia for these bone marrow aspirations. The side effects of the biopsies include pain, bleeding and infection. The bone marrow aspirations are scheduled as part of the transplantation protocol to keep track of your bone marrow recovery. No extra bone marrow aspirations will be done because of the gene marking protocol while you are hospitalized, but an extra one teaspoon of marrow will be taken during the exam to test for the MDR-1 . You will be asked to return monthly to NCI for evaluation after you are discharged from the hospital. Four extra teaspoons of blood will be drawn from you each month and a bone marrow aspiration will be obtained every three months to determine whether your bone marrow cells contain the MDR-1 gene. After six months you will return to NCI every two months at which time 4 teaspoons of blood and a bone marrow biopsy will be obtained if your bone marrow cells contain the MDR-1 gene. No further bone marrow biopsies will be obtained if the MDR-1 gene cannot be found on two successive bone marrow biopsies after you are discharged. We request that you return periodically to the NCI for the rest of your life so that we may follow you for any side effectsthat may occur. Should you die, for any cause, we will request that an autopsy be performed to study whether the MDR-1 gene is present in your body.

You will be asked to undergo a biopsy of your tumor prior to bone marrow

transplant to test it for drug resistance markers. If your tumor relapses or is found in a new part of your body after bone marrow transplant, a biopsy of this tumor will be obtained under local anesthesia to look for the presence of the MDR-1 gene in the tumor cells. The risks from these biopsies include pain, bleeding, infection or a punctured lung.

After the bone marrow transplantation you may be asked to undergo apheresis to obtain blood cells to study whether the MDR-1 gene is inside them. This procedure allows for the collection of large number of white cells without removing a lot of blood, since most of what is withdrawn is put back. Furthermore, all of the blood that is returned is your own; you will not receive the blood of another individual. The entire procedure takes one to two hours and will be performed in the Apheresis Center of the NIH Blood Bank. Adverse reactions to the apheresis procedures are rare. They include pain and rarely fainting due to transient lowering of the blood pressure. Tingling sensations around the mouth, fingers or toes and mild muscle cramps may occasionally occur as a side effect of the blood thiner used during the procedure. These symptoms are easily treated by stopping the procedure or giving Tums (calcium containing tablets). This procedure may be performed on more than one occasion, depending on how long the MDR-1 gene remains in your cells.

RISKS AND TOXICITIES

No side effects from the MDR-1 marker gene and the non-infectious retroviral vector carrying the MDR-1 gene virus to be used in this protocol have been seen in animal studies. However, since this is a very new procedure, the risks may not be known. There are some theoretical risks to this procedure. First, even though the virus used to insert the gene into your bone marrow cells cannot grow and is considered harmless, it is possible that events could occur within the cells that allow the virus to grow or cause the cell to become cancerous. It is also theoretically possible that a viral infection could set up in your body. If this should occur, it is possible that you could develop a malignancy. We do not expect this to happen because we monitor the transporter virus closely to make
sure that no infectious virus is present. It is possible that the cells containing the MDR-1 gene could delay your bone marrow recovery. We believe this is unlikely because enoughnormal marrow will also be given back to you to allow for normal recovery, but we cannot be sure. The special procedure that we will use to isolate your stem cells before exposing them to the MDR-1 gene may leave behind a small

amount of antibody on your cells. It is possible that this antibody could cause an allergic reaction. Over 50 patients have received stem cells purified using this method without any evidence of an allergic reaction, but you will be monitored for an allergic

reaction when you receive your cells back, and if one occurs, your physician will treat you for it.

It is possible that breast cancer cells that may be present in small numbers in your bone marrow or blood cells may also be infected with the MDR-1 gene. We will study whether this is the case by biopsying your tumor should it come back after transplant. We do not believe that you would be harmed should a breast cancer cell receive this MDR-1 gene; however, little information exists about this. It is possible that the MDR-1 gene could alter the breast cancer cell making it more or less malignant or more resistant to drugs such as vinblastine, taxol and doxorubicin. Should this happen, there are drugs to treat breast cancer that are not affected by the MDR-1 gene. It is also possible that the growth factors used to put the MDR-1 gene into the bone marrow or blood cells could cause breast cancer cells to grow. We believe that these possibilities are unlikely to cause you any harm if they did happen, but we can't be sure.

Participation in this study is entirely voluntary. You may choose to participate or you may withdraw from the gene transfer study at any time up to the time of infusion of the bone marrow. Enough bone marrow has been harvested to be able to proceed with the transplantation even without the cells that are treated with the MDR-1 gene. If you withdraw your consent, the gene-marked cells will not be infused. In the event that you do withdraw your consent after harvest, but do go ahead with transplantation, the course of your disease will be followed on a regular basis at NIH. You are encouraged to ask any questions that you may have and to take as much time as you need to make your decision.

The costs of hospitalization, treatment, and laboratory procedures related to the bone marrow transplantation that are provided at NIH are also paid for by NIH. It is important to understand that NIH cannot pay for tests or medical care that you receive outside of NIH.

All contact with the media will be coordinated through the National Cancer Institute's Office of Cancer Communications. We ask you to refer any journalists or other media personnel to Dr. O'Shaughnessy should you be contacted.

MEDICAL RECORD	CONTINUATION SHEET for either: NIH 2514-1, Consent to Participate in A Clinical Research Study NIH 2514-2, Minor Patient's Assent to Participate in A Clinical Research Study

STUDY NUMBER_____Continuation: page 6 of___ pages

OTHER PERTINENT INFORMATION

Confidentiality. When results of a study as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of National Institutes of Health or Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any physical injury resulting from your participating in research here. Neither the National Institutes of Health, Clinical Center nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.

Payments. If you are a patient, you are not paid for taking part in National Institute of Health studies. Exceptions for volunteers will be guided by the National Institutes of Health or Clinical Center policies.

Problems or Questions. Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator_____, or these other staff members also involved in this study: _____;_____;_____:
Building_____, Room_____. Telephone (301)_____.
National Institutes of Health; Bethesda, Maryland 20892

Consent Document. It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A OR B:

A. Adult Patient's Consent
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

_____
Signature and Date Signed

_____
Signature of Witness & Date

B. Parent's Permission for Minor Patient
I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study. (Attach NIH 2514-2, Minor's Assent, if applicable).

_____
Signature of Parent and Date Signed

_____
Relationship if other than Parent

_____
Signature of Witness & Date

PATIENT IDENTIFICATION	CONTINUATION SHEET FOR EITHER: NIH-2514-1 (9-91) NIH-2514-2 (9-91) P.A.:09-25-0099

Curriculum Vitae

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1985 - 1988 Fellowship	National Cancer Institute Medical Oncology

Board Certification:

1982	Diplomate, National Board of Medical Examiners
1985	Diplomate, American Board of Internal Medicine
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1986 - 1987	Post-doctoral fellow Laboratory of Tumor Virus Biology National Cancer Institute
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HUMAN GENE TRANSFER PROTOCOL

9309-055 Kun, Larry E.; Sanford, R. A.; Brenner, Malcolm K.; and Heideman, Richard L.; St. Jude Childrens Research Hospital, Memphis, Tennessee; and Oldfield, Edward H.; National Institutes of Health, Bethesda, Maryland; *Gene Therapy for Recurrent Pediatric Brain Tumors.*

Date of RAC Approval: 9/9/1993
Date of NIH Approval: 10/7/1993

GENE THERAPY FOR RECURRENT PEDIATRIC BRAIN TUMORS

Brain tumors are the second most common cancer in children. Supratentorial malignant gliomas account for 10-12% of childhood brain tumors. Although short-term survival appears to be superior to adults with malignant gliomas, no more than 20% of children with these tumors survive beyond 3 years. Associated supratentorial embryonal tumors (pineoblastomas, cerebral neuroblastomas, supratentorial PNET's) comprise an additional 5% of brain neoplasms in children, demonstrating survival rates similar to the malignant gliomas. The current proposal will study the feasibility of utilizing a novel approach of gene transfer therapy in children with recurrent or progressive malignant supratentorial tumors.

Building upon the preclinical and phase I adult studies at NIH (Dr. Edward Oldfield, NINDS), a phase I trial will utilize NIH 3T3 producer cells to deliver a retroviral vector (G1TKSVNa) to transfect proliferating brain tumor cells with the ganciclovir-susceptibility gene associated with the herpes simplex enzyme thymidine kinase (HS-tk). The brain is felt to be the ideal anatomic location for gene transfer based upon the selective proliferating capacity of the neoplastic cells (in contradistinction to the post-mitotic parenchymal brain cells) and relative "immunologic privilege" allowing exposure of the producer cells for vector integration. Subsequent delivery of ganciclovir has been shown to produce selective cell kill of the transfected neoplastic cells.

A phase 1 trial in children (3-21 years old) will allow assessment of feasibility and potential unique toxicities following the phase 1 adult study at NINDS. The producer cells will be instilled via multiple stereotactically placed cannulas, preplanned to homogeneously deliver a total of between 1 x 108 and 2 x 109 cells within the tumor volume. Seven days following the operative procedure, the patient will start a 14 day course of ganciclovir administration. Patients will be monitored for CNS, hematologic, renal, or other toxicities. Measurement of tumor response will include serial MRI studies, including an investigational analytical assessment of dynamic enhancement that appears to correlate with metabolic potential, and MR spectroscopy, an evolving technique correlating measured biochemical changes with tumor viability.

A total of 6 patients will be accessioned to the phase 1 study to allow adequate evaluation of potential toxicities. Guidelines include both parental consent and the child's assent. The investigators propose to follow the current trial with phase II studies including (1) traditional tumor type-specific evaluation of response in children with recurrent malignant supratentorial tumors, and (2) an "upfront" phase II study in children with supratentorial malignant gliomas.

APPENDIX D.

DESCRIPTION OF PROPOSED STUDY

Non-Technical Abstract

GENE THERAPY FOR RECURRENT PEDIATRIC BRAIN TUMORS

In an attempt to improve the grim prognosis associated with malignant pediatric brain tumors, a novel therapeutic approach to these tumors is being investigated. This approach uses recombinant DNA technology to transfer a gene which confers sensitivity to an antiviral drug into the tumor. This is achieved by direct injection of the tumor with cells which actively produce a retroviral vector carrying the sensitivity gene. The agent of transfer, or vector, is a retrovirus which has been modified so that it cannot reproduce itself. Such vectors are capable of stably incorporating their genetic material into the DNA of the host. The producer cell is an NIH 3T3 (mouse fibroblast) cell that has been genetically engineered to continually produce retroviral vectors. The vectors are inserted into dividing cells. Since normal brain cells are not actively growing or dividing, insertion of the producer cells into a brain tumor will result in vectors entering only the proliferating tumor cells. The vectors incorporate their DNA into the tumor cells, including the drug sensitivity gene. The gene leads the tumor cells to express a protein (herpes simples virus enzyme thymidine kinase or HS-tk) that sensitizes the cells to an antiviral drug (ganciclovir or GCV). When GCV is administered systemically, cells expressing the HS-tk protein convert GCV into a toxic substance intracellularly that leads to the death of the affected cells. Normal human cells inside and outside the brain are not sensitive to GCV. The selective uptake of the vector, therefore, allows specific cell kill of the affected tumor cells while sparing the adjacent normal brain cells and the cells outside the brain. This type of gene transfer has several unique features. First, these retroviral vectors only integrate and express their genes in cells which are actively synthesizing DNA. The brain in unique because normal brain cells are not mitotically active in children beyond 2 years of age. Therefore, only the actively dividing tumor cells are expected to express the HS-tk enzyme and become target for GCV; surrounding non-proliferating normal brain tissue should not acquire the HS-tk gene and will remain insensitive to GCV. Second, all of the transduced tumor cells (and retroviral vector producing cells) will be killed by the host immune response and/or GCV treatment, eliminating potential concern about vector-related changes giving rise to malignant cells in the tumor region or elsewhere.

SJCRH
CNS 18
July 1993

Protocol

GENE THERAPY FOR PEDIATRIC BRAIN TUMORS

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ELIGIBILITY CHECKLIST

Yes	No	
__	__	≥ 3 years and ≤ 21 years
__	__	RECURRENT PRIMARY MALIGNANT SUPRATENTORIAL GADOLINIUM ENHANCING BRAIN TUMOR without spinal tumor or positive CSF cytology.
__	__	≥3 Months after completion CNS irradiation
__	__	Histologic diagnosis previously established
__	__	Refractory to conventional treatment
__	__	Measurable supratentorial tumor accessible for multiple stereotactic cannula insertions
__	__	Serum Creatinine ≤2 x N or creatinine clearance ≥80 ml/min/m^2
__	__	Coagulation profile within normal range
__	__	Absence of any acute viral, bacterial or fungal infection requiring specific therapy
__	__	HIV Negative
__	__	Not pregnant if applicable
__	__	Platelet count ≥ 100,000/mm^3 and absolute neutrophil count ≥1,000/mm^3
__	__	Grade III/IV major organ dysfunction due to prior therapy absent.
__	__	Karnofsky score >40
__	__	Life expectancy ≥2 months
__	__	Informed Consent Obtained

All answers to above questions must be YES

__	__	Increased intracranial pressure is present.
__	__	Tumor is in direct contact with ventricular or cisternal spaces

All answers to the above questions must no NO

1.0 OBJECTIVES

This is a collaborative Phase I trial whose goals are:

1.1 To determine the safety of the implantation of xenogeneic vector-producing cells in pediatric patients with recurrent primary supratentorial malignant tumors.

1.2 To determine if ganciclovir administration is associated with a diminution in size of brain tumors following HS tk gene transduction.

2.0 BACKGROUND

2.1 Précis

Malignant brain tumors are responsible for significant morbidly and mortality in both pediatric and adult populations. The common malignant supratentorial tumors in children, including malignant gliomas and embryonal tumors, are an enormous therapeutic challenge. Despite radical surgery, high dose radiotherapy and, in some cases, chemotherapy, outcome remains poor. Survival of most patients from the time of diagnosis is measured in months; recurrence is associated with a life expectancy of weeks.

In an attempt to improve this grim prognosis of patients with malignant brain tumors, a novel approach is proposed in pediatric patients as part of a collaborative clinical trial being done in adults by investigators from the Surgical Neurology Branch, NCI and NIH. If this approach is successful, we will succeed in incorporating a gene conferring drug sensitivity into brain tumor cells. This will be done by direct injection of a producer cell line which actively produces a retroviral vector carrying the desired drug sensitivity gene, HS-tk.

The producer cell is an NIH 3T3 (mouse fibroblast) cell that has been genetically engineered to produce transducing retroviral vector continually. Such vectors are capable of transferring foreign DNA into mammalian cells. The vector to be used in this study has been engineered to incorporate the new genetic material stably into the genome of the transduced host cell. The protein encoded by the new gene is the herpes simplex virus enzyme thymidine kinase, HS-tk, which sensitizes actively dividing tumor cells to an antiviral drug (ganciclovir, GCV) which is a substrate for the HS-tk. Ganciclovir is then given in an attempt to kill all transduced tumor cells. The form of the thymidine kinase enzyme normally present in mammalian cells has very low affinity for GCV. Therefore, only cells transduced by the vector and expressing the viral HS-tk gene are expected to be killed by GCV. Systemic toxicity is not expected. This type of *in vivo* gene transfer has several unique features. First, these retroviral-vectors were engineered to integrate and express the genes they carry only in cells actively synthesizing DNA. Therefore, surrounding non-proliferating normal brain tissue present in those over 3 years of age should theoretically not acquire the HS-tk

gene and remain insensitive to the effects of GCV. Second, all of the transduced tumor cells (and retroviral vector producing cells) should be killed by the host immune response and/or GCV treatment eliminating potential concern about insertional mutagenesis giving rise to malignant cells.

This is the first clinical proposal to treat malignant pediatric CNS tumors by *in - vivo* genetic manipulation of the tumor's genome.

2.2 Clinical Background

Brain tumors are a major cause of morbidity and mortality with approximately 35,000 new cases in all age groups each year in the U.S. They comprise the third leading cause of death from cancer in persons 15-34 years of age, and may be increasing in prevalence in some populations (1, 2). The astroglial brain tumors, including the highly malignant glioblastoma multiforme (GBM), are the most common primary brain tumors in adults. Despite aggressive therapy which includes surgical removal of the tumors and post-operative high dose radiation therapy, the prognosis for patients with GBM is very poor with a median survival of 9-10 months.(3) Although controversial, it appears that neither the quality nor the interval of survival is significantly improved when chemotherapy is added to surgery and radiation (4). When GBM recurs, there is a 100% mortality within weeks to a few months. In one study, a mean survival of only 36 weeks was found in patients with recurrent GBM who underwent a second operation with a reasonable quality of life limited to 10 weeks (5).

CNS tumors are the second most frequent malignancy in children under the age of 15 years (6). About 45% of children with CNS tumors die from their disease. In children, malignant supratentorial astroglia tumors account for 10-12% of brain tumors. Although children have longer survival intervals than adults, (18-24 months compared to 8-10 months), fewer than 20% remain alive at 3 years. When compared to adults, pediatric malignant gliomas have a higher propensity for leptomeningeal dissemination. The dominent pattern of failure remains local recurrence, present in 90% of cases (7-9).

The supratentorial embryonal tumors (pineoblastoma, cerebral neuroblastoma and primitive neuroectodermal tumors) account for 4-5% of childhood brain tumors; 20% 2-year disease free survival with current combined modality therapy is the rule. Patients with recurrent disease are rarely salvaged, and quickly die of their disease (7,10,11).

2.3 Proposed Retroviral vector-mediated Treatment of Human Brain Tumors

The central nervous system has several biologic properties which make it appropriate for the study of in-vitro gene transfer therapy. First, the retroviral vectors to be used only integrate and express genes in proliferating cells. In adults and children over the age of 2 years, tumors comprise the most mitotically active group of cells in the CNS; macrophage-derived cells, blood cells and endothelial

cells maintain some mitotic activity, and may be at some (minimal) risk for viral incorporation. Therefore, the likelihood of specific transduction of desired gene into tumor cells vs other cells is enhanced. Second, the brain is a partially immunologically privileged site which should allow a somewhat longer survival of the xenogeneic murine cells in the brain, leading to greater virus production *in situ* and thus potentially a greater transduction efficiency than might be seen in other organs. This and the ability of many CNS tumors to depress local immunity (12) should allow for the transduction of greater numbers of tumor cells. By design, this period of survival will be limited as cells that integrate and express the HS-tk gene, including the retroviral vector producer cells, should later be destroyed by GCV.

2.3.1 Gene Transfer Methods

There are 3 major methods of gene transfer: 1) chemical, 2) physical and 3) viral (e.g. herpes simplex, adenovirus, retrovirus) (13). Physical and chemical methods have poor gene integration efficiency, ranging from 1:1,000, to 1:100,000, making them impractical for most clinical applications. The adenovirus and herpes simplex viruses have the ability to replicate extra-chromosomally, and have been used as vectors to transfer genes into cells where they replicate in an extrachromosomal location. Therefore, these viruses, unlike the murine retrovirus from which our vector is derived, can transfer genes into non-replicating tissues (e.g. lung, brain).

2.3.2 Retroviral-Mediated Gene Transfer (14)

Murine retroviral vectors are proven extremely efficient for gene transfer into mammalian cells - as high as 90% in cultured murine fibroblast line(s). Murine retroviral vectors integrate and subsequently express their genes at high efficiency only in proliferating cells. Because tumor cells are the predominant mitotic cell in the otherwise post-mitotic CNS, differential transduction of tumor should be maximal while transduction into brain parenchymal cells is minimal or absent. The moloney murine leukemia virus-based (MoMLV) vectors to be used in the current protocol have been designed to minimize the possibility of recombination leading to regeneration of replication-competent virus (15,16).

2.3.3 Experience with Retroviral-mediated Gene Transfer Into Humans
N2/TIL Marking Study:

In 1989, the first gene transfer experiment in humans was conducted at the NIH. This study involved the treatment of 10 patients with autologous T-cells (TIL) transduced with the

retroviral vector LNL6 (constructed by A. Dusty Miller; supernatant produced by Genetic Therapy, Inc). The G1TKSVNa vector to be used in the current protocol is modelled on the LNL6, having the same general structure and safety modification. None of the patients have demonstrated any untoward effects since receiving the genetically-altered cells (17).

Human Gene Therapy for Adenosine Deaminase Deficiency (ADD)

Since September, 1990, two children have been enrolled on a protocol treating adenosine deaminase associated (ADA) immune deficiency. Fifteen intravenous infusions of autologous T-cells transduced by the LNSN vector have been administered to these patients and significant improvement of their severe immunodeficiency has occurred. The LASN vector (constructed by A. Dusty Miller; supernatant produced by Genetic Therapy, Inc.) is based on the LNL6 vector and therefore has the same general structure and safety modifications as the G1TKSVNa vector proposed for use in the current protocol. Neither child has demonstrated any evidence of adverse effects due to the genetically altered cells.

Other Human Gene Therapy Experiments

A number of other gene-marking experiments (such as those being done currently at St. Jude in patients with acute non-lymphoblastic leukemia and neuroblastoma) as well as therapeutic experiments have been underway for over 18 months. More than 80 patients have been treated. No untoward side effects related to retroviral mediated gene transfer have been observed in any individual.

The above experiences with retroviral-mediated gene transfer suggests that therapeutic approaches using this technology are reasonably safe, and may possess efficacy. The experiences outlined above and the poor prognosis of recurrent malignant pediatric CNS tumors support initiating a phase I trial to evaluate the safety of retroviral vector therapy in pediatric patients with recurrent brain tumors.

2.4 Preclinical Retroviral HS-tk Gene Therapy Studies

Investigators of NINDS, NIH have performed a number of pre-clinical studies which demonstrate that retroviral transduction of xenogeneic genes can be done efficiently. Safety and antitumor efficacy have been shown in rodents and non-human primates. Due to the large amount of such information, all of which has been previously reviewed by the RAC, it is summarized in Protocol Appendix I.

3.0 DRUG, VECTOR, GENE, PRODUCER CELL LINE INFORMATION

3.1 Ganciclovir (GCV, Cytovene)

Source: Commercially available (Syntex corporation, Palo Alto, CA)

Pharmacology: Ganciclovir is a synthetic nucleoside analogue of 2'deoxyguanosine that inhibits replication of herpesviruses both in vitro and in vivo. Sensitive human viruses include cytomegalovirus, herpes simples virus types 1 and 2, varicella zoster virus. Evidence indicates that upon entry into host cells, these viruses induce cellular kinases that phosphorylate ganciclovir to its triphosphate. Ganciclovir is believed to inhibit viral DNA synthesis by direct competitive inhibition of viral DNA polymerases and direct incorporation into viral DNA resulting in eventual termination of DNA chain elongation.

GCV does cross the blood-brain-barrier. The cerebrospinal fluid to plasma ratio is from 0.24 to 0.70 (0.31-0.68 ug/ml in CSF and 0.44-2.2 ug/ml in plasma). The above CSF and plasma concentrations are expected to be within the range of GCV needed to kill the HS-tk transduced cells based on in vitro and pre-clinical in vivo studies (0.5 ug/ml will prevent growth of HS-tk transduced tumor cells).

Formulation and Stability and Route of Administration: Sterile powder is supplied in 10 ml vials, each containing ganciclovir sodium equivalent to 500 mg of ganciclovir. Vials may be stored at room temperature, and the drug is stable at temperatures less than 40° C. Ganciclovir is reconstituted with 10 ml of sterile water for injection. This results in a solution of 50 mg/ml ganciclovir. **Note: Do not use bacteriostatic water containing parabens for injection. It is incompatible and may lead to drug precipitation.** The reconstituted drug is stable at room temperature for 12 hours, after which it must be discarded. It should not be kept refrigerated. Prior to administration, the appropriate amount of drug should be further diluted with 0.9% saline to a final concentration of 10 mg/ml or less. Ganciclovir is to be infused intravenously over 1 hour. Do not give as a rapid or bolus administration. Because non-bacteriostatic saline is used for reconstitution, ganciclovir should be used promptly after reconstitution. Solutions greater than 12 hours old should be discarded.

Toxicity: Hematologic toxicity, dominantly thrombocytopenia and granulocytopenia have been seen with a frequency of up to 32% in immunosuppressed patients with AIDs, transplant recipients, or patients with bone marrow allografts. In non-immunosuppressed patients the frequency of such events is not greater than that associated with placebo. Hematologic toxicity appears transient and generally responds to withdrawal of ganciclovir. Renal toxicity, consisting of transient and reversible increases in serum creatinine to greater than 2.5 mg/dl may occur in up to 18% of patients during drug administration, and may require dose modification. Transient complaints of headache and confusion may also occur.

3.2 The G1TKSVNa Retroviral Vector

Source: Genetic Therapy Incorporated, Rockville, MD.

Pharmacology/Virology: G1TKSVNa is a retroviral vector derived from the Moloney murine leukemia virus (MoMLV). This vector contains a herpes thymidine kinase (HS-tk) gene cDNA that is transcribed from the viral LTR and a bacterial neomycin resistance (NeoR) gene transcribed from an internal SV40 (simian virus 40) early promoter (LTR-HS-tk-SV-NeoR-LTR) in the G1 vector backbone (Genetic Therapy Inc., Gaithersburg, MD). This G1-based vector has been modified for increased safety by alteration of the gag start codon to a stop codon, and by elimination of viral sequences needed for the formation of the virus. This has been shown to minimize the potential for helper virus production from producer cells which contain the vector. No replication-competent virus has been detected during long term culture or following administration of the vector to animals or humans.

3.3 HS-tk Gene

The herpes simplex thymidine kinase (HS-tk) gene is a negative selectable marker or "suicide" gene. When a HS-tk transduced cell is exposed to ganciclovir (GCV), the GCV acts as a substrate for phosphorylation by HS-tk resulting in a triphosphate (TP) form of the drug. This phosphorylated form (GCV-TP) inhibits DNA polymerase and is incorporated into DNA resulting in an inability of the cell to proliferate. The end result is cell death for the HS-tk transduced cells. The NeoR gene is a positive selectable marker. The bacterial NeoR gene encodes for NPTII (neomycin phosphotransferase II), an enzyme that will protect G1TKSVNa expressing cells from the toxic effects of G418 (a neomycin analog). NeoR is widely used in our retroviral vectors and has been used in all human clinical trials to date without adverse effect. NPT-II inactivates the antibiotic Amikacin but does not inactivate other aminoglycoside antibiotics (such as gentamicin and tobramycin). The introduction of the NeoR gene should not affect the clinical management of gram negative infections in the patients.

3.4 Producer Cell Line

3.4.1 Preparation of the G1TKSVNa-Producer Cell Line

The vector construct was transfected into the PA317 (ATCC CRL 9078) packaging line cells. The generation of retroviral vectors from transinfected PA317 cells has been extensively tested in vitro and in human gene transfer/therapy experiments and all clinically certified preparations are free of replication-competent retrovirus. Further details are included in Protocol Appendix II.

The transinfected G1TKSVNa cells were selected in G418 and cloned. Clone 90 has produced the highest NeoR and HS-tk titer (5×10^5 cfu/ml

for both) and this clone has been approved by the FDA for use in our clinical studies. All batches of the G1TKSVNA-producer cell lines used for injection into humans will have met FDA specifications for current clinical human gene therapy experiments in progress.

For use in the current study, frozen and certified cell lines will be shipped to Memphis and thawed immediately prior to use (see Appendix A).

4.0 ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

1. $\geq$ 3 years and $\leq$ 21 years

2. PROGRESSIVE OR RECURRENT SUPRATENTORIAL GADOLINIUM ENHANCING BRAIN TUMOR OF ANY MALIGNANT HISTOLOGY, without lepto-meningeal tumor as documented spinal MRI and CSF cytology (when possible).

3. Patients must be at least 3 months beyond completion of radiation therapy, and recovered from the acute toxicities associated with any prior chemotherapy.

4. Histologic diagnosis previously established.

5. Minimum tumor size 1.5 cm^3 ; maximum tumor size 5 cm^3.

6. Serum Creatinine $\leq$ 2 x N or creatinine clearance $>$ 80 $ml/min/m^2$

7. Coagulation profile within normal range

8. Absence of any acute viral, bacterial or fungal infection requiring specific therapy

9. HIV Negative

10. Not pregnant

11. Platelet count $\geq$ 100,000/mm^3 and absolute neutrophil count $\geq$1,000/mm^3

12. Absence of any grade III/IV major organ dysfunction

13. Karnofsky score $>$40

14. Life expectancy $\geq$ 2 months

15. Informed Consent Obtained

4.2 Exclusion Criteria

1. Acutely increased intracranial pressure (ICP) requiring prompt surgical (shunt, tumor resection) reduction.

2. Tumors in direct contact with the ventricular or cisternal spaces (brain stem tumors, spinal cord tumors, superficially located cerebellar tumors).

5.0 STUDY DESIGN AND TREATMENT

This is a nonrandomized, Phase I toxicity study. Therapeutic intent is implied, and responses to treatment will be accessed.

Patients who exhibit a partial response to therapy and no significant, irreversible toxicity, will be considered for repeated treatments based on their clinical status.

5.1 Stereotactic Delivery of Producer-Vector Cells

Multiple CR or MRI-guided stereotactic injections of the HS-tk vector-producer cells into the brain tumor tissue will be done under general anesthesia. The number of injection points will be preplanned and determined by the size of the tumor. Each injection will be done using a volumetric pump over 10-30 minutes duration.

5.1.1 Volume and Number of Injected Cells

The tumor size, shape, and location will determine the number and spacing of stereotactic injections. The cannula placement will be targeted at roughly 3 mm spacing homogeneously within the tumor volume. The producer-vector cell concentration is 1×10^8 cells/ml; between 1×10^8 and 2×10^9 cells will be administered within the tumor.

5.1.2. Disposal of residual producer cells.

Residual cells and the accompanying vial will be placed in an infectious waste container and autoclaved before disposal.

5.2 Peri-Operative Medications

5.2.1 *Antibiotics*: All patients will receive a single dose of Ceftriaxone (2 g/m^2 IV) just prior to the surgical procedure.

5.2.2 *Steroids*: Dexamethasone (16 mg/m^2/day in 3 divided doses) will be started 3 days prior to the stereotactic injection of vector-producer cells and continued until GCV is discontinued. Dexamethasone will then be

tapered according to individual patient requirement. As experience is gained, the need for high dose dexamethasone may be modified.

5.2.3 *Mannitol*: 1 g/kg will be given during surgery and every 8 hours for a total of 24 hours following the procedure.

5.2.4 *Anticonvulsants*: Anticonvulsive therapy will be administered according to the usual neurosurgical guidelines for such medications.

5.2.5 *Analgesics*; Pain medications will include Acetaminophen 5-10 mg/kg/dose q4 hours (max dose 1000 mg QID).

5.3 Ganciclovir Sodium Administration

On day #7 following instillation of producer-vector cells, GCV will be administered by intravenous infusion over one hour. A dose of 5 mg/kg of body weight is to be given twice daily (10 mg/kg/day total dose) for 14 consecutive days. Ganciclovir is reconstituted with sterile water and diluted with normal saline to a final concentration of 10 mg/ml.

5.3.1 GCV Dose Interruption for Toxicity

A total of 14 days of ganciclovir is to be given; if therapy is delayed (see below), more than 14 days may be needed to deliver all 14 scheduled doses. Do not omit doses.

5.3.1.1 Hematologic

The development of an ANC of <500 cells/mm^3 or a platelet count of $<25{,}000$/mm^3 require dose interruptions until the ANC is ≥ 750 cells/mm^3 and platelet count is $\geq 40{,}000$ platelets/mm^3. Call study Principal investigator if hematologic toxicity persists >3 days.

5.3.1.2 Renal

Renal toxicity has primarily been reversible increase in serum creatinine levels. No dose modifications will be made for serum creatinine level below 2.5 x N. If the serum creatinine increase is greater than 2.5 x N for age, delay further GCV until creatinine level has fallen to less than 1.5 N for age. Call study Principal investigator if renal toxicity persists greater than 3 days.

5.4 Supportive and Concurrent Care

5.4.1 Fever and neutropenia

In the event of the development of fever and neutropenia or significant bacterial infection (such as central line infection or ventriculoperitoneal shunt), antibiotic combination of first choice is vancomycin and ceftazidime. Nephrotoxic antibiotics or antifungal agents may increase the incidence of renal toxicity associated with GCV. Their use should be dictated by clinical circumstances including surveillance cultures and institutional infection patterns. Do not stop ganciclovir unless the criteria in Section 5.3.1 are present.

5.4.2 Fever and chills with ganciclovir should be treated with acetaminophen every 4 hours at age appropriate dosages.

5.4.3 Rashes should be treated with diphenhydramine 25 mg/m^2 q6 hours IV or po.

5.4.4 Severe edema - as a precaution, all patients will be treated with decadron as noted in Section 5.2.2.

5.4.5 All patients should be on trimethoprim-sulfamethoxazole prophylaxis (150 mg/m^2/day based on trimethoprim) Monday, Tuesday, Wednesday, po bid.

5.4.6 Venous access must be adequate. A percutaneous intravenous catheter can be used if a central line or port-a-cath is not present.

6.0 REQUIRED STUDIES

	Pre-Surgical	Stereotactic surgery	Ganciclovir Therapy	Mo 1-2	Mo 3-15
Height, weight	X		Pre		q 3 mo
Karnofsky	X	Pre, post	Pre, end		q 3 mo
Physical exam	X	Pre, post	Pre, end	q2 wk	
Neurologic evaluation	X	Daily	Daily	q2 wk	q mo
MRI brain (DEMRI and static Gd contrast)	X	within 48 hr post-op	Pre & day 14	q mo	q 3 mo
*proton MRS, brain	X		Day 14	q mo	q 3 mo
Phosphorus MRS, brain	X		Pre & day 14	q mo	q 3 mo
MRI spine, contrast	X				
CSF - cells, protein, glucose	X**		End	After 1 mo	
CBC, differential, platelet	X		2-3 x wk	q2 wk	q 3 mo
Urinalysis	X				
BUN, creatinine	X		2-3 x/wk		
Creatinine clearance	X				
Bilirubin, TP, aluminum, GOT, GPT, alkaline phos	X		Weekly		
Coagulation profile	X				
Electrolytes	X				
Ca, P, uric acid	X				
Anticonvulsant levels	X (if used)		Pre		

* This study will be performed as part of MRI brain evaluation, above, and will not require separate examination or sedation.

** If clinically safe based on patients examination, MRI and in the absence of papilledema.

6.1 Neuroimaging Evaluation

MRI, including dynamic contrast-enhanced MR imaging (DEMRI), will be performed to access tumor response and to monitor for imaging evidence of neurotoxicity (inflammation, hemorrhage). DEMRI is an investigational analytical assessment of metabolic activity in and around the tumor-bone upon the time-related appearance of contrast. See Protocol Appendix III.

Magentic resonance spectroscopy (MRS) is a rapidly evolving science that correlates measurable biochemical concentrations with metabolic changes within treated tumors and peritumoral brain. See Protocol Appendix III.

6.2 Yearly Follow-up Laboratory Evaluation

In addition to standard and investigational studies, patients will be followed once a year for 14 years for retroviral gene transfer safety monitoring. Studies will include CBC with differential and platelet count, and PCR on mononuclear cell DNA for vector sequences (2 ml heparinized peripheral blood. Send to GTI, Gaithesburg, MD.)

6.3 Autopsy

An attempt will be made to perform a complete autopsy on any patient who dies during the study. Whenever possible, tissues from the brain, tumor(s), bone marrow, will be evaluated for the presence of the G1TKSVNa vector by PCR. Such specimens will be frozen at -70° and sent to GTI, Gaithersburg, MD. Whenever the autopsy is performed outside SJCRH, an attempt will be made to obtain tissue and pathological slides of the CNS for review.

7.0 EVALUATION CRITERIA

NO RESPONSE:	$<50\%$ decrease in size or progression of the tumor by MRI scan.
PARTIAL RESPONSE:	$\geq 50\%$ and $<100\%$ decrease in tumor volume on MRI scan.
COMPLETE RESPONSE:	No remaining tumor on MRI at 3 months.

8.0 TOXICITY MONITORING

Toxicity will be monitored and graded according to the NCI Common Toxicity Criteria (see attached). Unacceptable toxicity is defined as a) any Grade III/IV major end organ toxicity and b) any irreversible cerebral dysfunction (motor, sensory, cognitive), or injury not clearly related to neurosurgery or not present prior to study enrollment.

Toxicity due to ganciclovir therapy and/or neurologic complications due to CNS tumor itself and/or its progression, will not be considered as unacceptable toxicity.

In the event of Grade III/IV major end organ toxicity or unique or unexplainable events, an adverse drug report (ADR) report will be filed. The St Jude Children Hospital IRB, and the principal investigator(s) at the Surgical Neurology Branch, NIH, will be notified.

9.0 OFF STUDY CRITERIA

9.1 Progressive disease at any time after treatment.

9.2 Refusal of further therapy by the patient or legal guardian.

9.3 Development of unacceptable toxicity or death on study. **Notify Principal investigator immediately.**

10.0 STATISTICAL CONSIDERATIONS

This is a feasibility/phase I (no dose escalation) clinical trial which has the primary objective of evaluating the toxicity associated with intratumoral transduction with the thymidine kinase gene and subsequent treatment with intravenous ganciclovir. The statistical design is adapted from the CTEP recommended design for phase I trials in pediatric oncology and requires the successfully study of at most six patients. Patients will be treated one at a time and the trial will be terminated when two toxic episodes have been observed or six patients have been treated with one or none having experienced unacceptable toxicity. The above design has 34%, 58%, and 89% chance of identifying the regimen as having unacceptable toxicity if the true toxic rate is 20%, 30% or 50, respectively.

If one or none the six evaluable patients experience unacceptable toxicity, the regimen will be considered feasible and permission for a disease specific phase II trial will be sought to investigate efficacy.

A total of 31 previously untreated patients who would ultimately have been eligible for this protocol were seen at SJCRH from January, 1989 to June, 1993. Based on this, 7 patients/year are expected to be eligible for this study. Furthermore, it is anticipated that the numbers of eligible patients may be substantially increased, if required, through accession of previously treated patients with recurrent tumors.

11.0 DATA MANAGEMENT/REPORTING REQUIREMENTS

Data including prior and current therapies, clinical status, laboratory results, neurologic and imaging evaluations, will be compiled and maintained by the data manager (L. Ogle). This information will be mailed to NCI investigators every 1-2 months as well as other monitoring agencies, when appropriate.

Adverse drug reactions are to be promptly reported to the Investigational Drug Branch, NCI, phone (301) 496-7957. These reports are required even if the event is only a suspicious for a drug effect. Previously unknown grade 1,2, and 3 reactions will be reported in writing using the NCI forms within 10 working days (Send to: Investigational Drug Branch, Cancer Therapy Evaluation Program, P.O. Box 30012, Bethesda, MD 20824). Grade 4 reactions, or deaths on study must be reported to the IRB within 24 hrs, with a written report to follow within 10 working days.

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SUMMARY STATEMENT (Chart Copy)
GENE THERAPY IN RECURRENT BRAIN TUMORS

You/your child has a brain tumor which has come back despite having had chemotherapy, radiation or both. The chance for a cure is now highly unlikely using conventional approaches such as surgery or other chemotherapy.

We are asking you/your child to take part in a new and very experimental approach. We hope to find out if a special gene (a piece of DNA) can be transferred into brain tumors, and if a drug can then be given so that the transferred gene can then be used to help kill the tumor. Surgery is used to place mouse cells that make a special mouse virus (one that can enter dividing cells but cannot cause disease) into the tumor. This special mouse virus carries a gene called thymidine kinase which comes from a human virus. Tumor cells, but not normal brain cells, take up the special virus and the new gene it carries. We think any cells which carry the new gene will be damaged or killed when you/your child are given an antivirus drug called ganciclovir. The mouse cells should also be killed by this drug. Because only the mouse cells and you/your childs tumor cells will carry this target, we think that normal brain cells will not be hurt by ganciclovir.

This approach has been used in animals and the results are encouraging. A few adults have also been treated in this way and have not been harmed. However, this is the first time that this experimental therapy is to be used in children.

Pretreatment Evaluation

You/your child will have a spinal tap to check for malignant cells, and magnetic resonance images (MRI) (of both the brain and spine) with and without a dye to make the picture clearer. A special procedure like MRI, called MR spectroscopy (MRS), will also be done at that time if you/your child agree. You/your child will also have blood tests including blood counts, liver and kidney function tests and blood chemistries. We will also do blood studies for HIV, the virus which causes AIDS. If you/your child are positive, we will not be able to give you/your child this treatment. In teenage women having regular menses a pregnancy test will be done. If this shows pregnancy, we will not be able to give you/your child this treatment.

TREATMENT

You/your child will have an operation during which the mouse cells that make the special virus are directly injected into the tumor. This operation will be done while you/your child is under general anesthesia. The neurosurgeon will guide a needle to the tumor location(s) through a hole drilled in the skull. We will use an MRI scanner and a computer to guide the injection and to try not to do damage to normal brain. The cells will be slowly injected. Several separate injections will be made to place the mouse cells into different areas of the tumor. The total number of injections will depend on the size of the tumor. Some tumors may require more than one hole in the skull. Following surgery, you/your child will be monitored in an intensive care unit.

You/your child will begin to receive ganciclovir (Cytovene) 7 days after surgery. This will be given over 1 hour, twice a day for 14 days. MRI scans will be done several times during the following days to weeks to see if the treatment is working.

All patients will be followed as an outpatient with serial blood tests, MRI scans and neurologic examinations done at each visit. At first, you/your child will be seen every two weeks for two months and then monthly for 10 months. Visits after the first year will be scheduled at 3-6 month intervals until 5 years, and then annually.

RISKS

The surgical risks depend on how well you/your child are before the operation, and the place and size of the tumor. All brain operations may cause bleeding or worsening of brain function (such as weakness in arm and/or leg, loss of sense of touch over parts of the body, loss of speech, ability to understand, memory). There is also risk of infection and even of death. Your/your childs particular risks will be discussed with you by the neurosurgeon.

There are also risks due to the gene therapy. Even though the special mouse virus is inactivated, cannot grow and is considered harmless in humans, it is possible that the virus may grow and/or make the normal cells cancerous. Since 1989, more than 80 people have had treatment with similar viruses and these problems have not occurred. But although we think these problems are unlikely, they might happen.

The special virus may also go into surrounding normal tissue and cause damage when you/your child get ganciclovir. This has not happened in any animal experiment or in the adults who have been treated so far.

Ganciclovir may cause side effects. The most common has been a fall in white blood cell and platelet counts which increases the risk of infection and bleeding. We will stop the ganciclovir if the blood counts drop too much. Ganciclovir can also cause permanent or temporary infertility and may be associated with birth defects. Therefore, men and women of child bearing age should use contraceptives during and for at least 90 days following ganciclovir therapy.

Following therapy, changes indicating a reaction to treatment may be seen on MR imaging. These images may be temporary or long lasting. The changes may be associated with temporary or permanent neurologic problems. Because this is an experimental treatment there may be other risks that we do not know.

BENEFITS

We hope, but we do not know, that you/your child will have a decrease in the size of the brain tumor and that the information gained will benefit others.

ALTERNATIVE TREATMENTS

You/your child may decline any further treatment. No standard treatment is known to be of benefit, but other experimental therapies are available at this and other institutions.

STATEMENT OF UNDERSTANDING

1. I have read the material above and I willingly agree to take part/let my child take part in this study.

2. I have been able to talk as much as I want to with Dr. _____, who is taking care of me or my child _____, about the reasons for this study and about its risks.

3. I have been told about the other choices for treatment or for no treatment.

4. I know that this study may have risks that the doctors do not know about now.

5. I know that I/my child can withdraw from this study at any time. If this happens, I/my child can still be treated at St. Jude.

6. I know that I/my child will not be paid for being in this study.

7. I know that if I am injured/my child is injured because of this study, I/my child will not get any money for being injured. However, St. Jude will treat the injury, free of charge.

8. I know that my/my child's records will not be given to anyone outside the hospital unless I agree. I agree that my/my child's records may be reviewed by agencies such as the Food and Drug Administration or the National Cancer Institute.

9. I know that if I have more questions about this study or about any injury from the treatment, I can call _____ at _____.

10. I know that I can get more information about my/my child's rights as a study participant by calling the Chairman of the Institutional Review Board at 901/522-0300.

11. I will receive a signed copy of this statement.

_____	_____
Patient	Parent
_____	_____
Physician	Witness
_____	_____
Date	Time

In case of questions or emergencies in reference to this protocol, please contact:
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105
(901) 522-0300

SUMMARY STATEMENT (Parent Copy)
GENE THERAPY IN RECURRENT BRAIN TUMORS

You/your child has a brain tumor which has come back despite having had chemotherapy, radiation or both. The chance for a cure is now highly unlikely using conventional approaches such as surgery or other chemotherapy.

We are asking you/your child to take part in a new and very experimental approach. We hope to find out if a special gene (a piece of DNA) can be transferred into brain tumors, and if a drug can then be given so that the transferred gene can then be used to help kill the tumor. Surgery is used to place mouse cells that make a special mouse virus (one that can enter dividing cells but cannot cause disease) into the tumor. This special mouse virus carries a gene called thymidine kinase which comes from a human virus. Tumor cells, but not normal brain cells, take up the special virus and the new gene it carries. We think any cells which carry the new gene will be damaged or killed when you/your child are given an antivirus drug called ganciclovir. The mouse cells should also be killed by this drug. Because only the mouse cells and you/your childs tumor cells will carry this target, we think that normal brain cells will not be hurt by ganciclovir.

This approach has been used in animals and the results are encouraging. A few adults have also been treated in this way and have not been harmed. However, this is the first time that this experimental therapy is to be used in children.

Pretreatment Evaluation

You/your child will have a spinal tap to check for malignant cells, and magnetic resonance images (MRI) (of both the brain and spine) with and without a dye to make the picture clearer. A special procedure like MRI, called MR spectroscopy (MRS) will also be done at that time if you/your child agree. You/your child will also have blood tests including blood counts, liver and kidney function tests and blood chemistries. We will also do blood studies for HIV, the virus which causes AIDS. If you/your child are positive, we will not be able to give you/your child this treatment. In teenage women having regular menses a pregnancy test will be done. If this shows pregnancy, we will not be able to give you/your child this treatment.

TREATMENT

You/your child will have an operation during which the mouse cells that make the special virus are directly injected into the tumor. This operation will be done while you/your child is under general anesthesia. The neurosurgeon will guide a needle to the tumor location(s) through a hole drilled in the skull. We will use an MRI scanner and a computer to guide the injection and to try not to do damage to normal brain. The cells will be slowly injected. Several separate injections will be made to place the mouse cells into different areas of the tumor. The total number of injections will depend on the size of the tumor. Some tumors may require more than one hole in the skull. Following surgery, you/your child will be monitored in an intensive care unit.

You/your child will begin to receive ganciclovir (Cytovene) 7 days after surgery. This will be given over 1 hour, twice a day for 14 days. MRI scans will be done several times during the following days to weeks to see if the treatment is working.

All patients will be followed as an outpatient with serial blood tests, MRI scans and neurologic examinations done at each visit. At first, you/your child will be seen every two weeks for two months and then monthly for 10 months. Visits after the first year will be scheduled at 3-6 month intervals until 5 years, and then annually.

RISKS

The surgical risks depend on how well you/your child are before the operation, and the place and size of the tumor. All brain operations may cause bleeding or worsening of brain function (such as weakness in arm and/or leg, loss of sense of touch over parts of the body, loss of speech, ability to understand, memory). There is also risk of infection and even of death. Your/your childs particular risks will be discussed with you by the neurosurgeon.

There are also risks due to the gene therapy. Even though the special mouse virus is inactivated, cannot grow and is considered harmless in humans, it is possible that the virus may grow and/or make the normal cells cancerous. Since 1989, more than 80 people have had treatment with similar viruses and these problems have not occurred. But although we think these problems are unlikely, they might happen.

The special virus may also go into surrounding normal tissue and cause damage when you/your child get ganciclovir. This has not happened in any animal experiment or in the adults who have been treated so far.

Ganciclovir may cause side effects. The most common has been a fall in white blood cell and platelet counts which increases the risk of infection and bleeding. We will stop the ganciclovir if the blood counts drop too much. Ganciclovir can also cause permanent or temporary infertility and may be associated with birth defects. Therefore, men and women of child bearing age should use contraceptives during and for at least 90 days following ganciclovir therapy.

Following therapy, changes indicating a reaction to treatment may be seen on MR imaging. These changes, may be temporary or long lasting. The changes may be associated with temporary or permanent neurologic problems. Because this is an experimental treatment there may be other risks that we do not know.

BENEFITS

We hope, but we do not know, that you/your child will have a decrease in the size of the brain tumor and that the information gained will benefit others.

ALTERNATIVE TREATMENTS

You/your child may decline any further treatment. No standard treatment is known to be of benefit, but other experimental therapies are available at this and other institutions.

STATEMENT OF UNDERSTANDING

1. I have read the material above and I willingly agree to take part/let my child take part in this study.

2. I have been able to talk as much as I want to with Dr. _____, who is taking care of me or my child _____, about the reasons for this study and about its risks.

3. I have been told about the other choices for treatment or for no treatment.

4. I know that this study may have risks that the doctors do not know about now.

5. I know that I/my child can withdraw from this study at any time. If this happens, I/my child can still be treated at St. Jude.

6. I know that I/my child will not be paid for being in this study.

7. I know that if I am injured/my child is injured because of this study, I/my child will not get any money for being injured. However, St. Jude will treat the injury, free of charge.

8. I know that my/my child's records will not be given to anyone outside the hospital unless I agree. I agree that my/my child's records may be reviewed by agencies such as the Food and Drug Administration or the National Cancer Institute.

9. I know that if I have more questions about this study or about any injury from the treatment, I can call _____ at _____.

10. I know that I can get more information about my/my child's rights as a study participant by calling the Chairman of the Institutional Review Board at 901/522-0300.

11. I will receive a signed copy of this statement.

_____	_____
Patient	Parent
_____	_____
Physician	Witness
_____	_____
Date	Time

In case of questions or emergencies in reference to this protocol, please contact:
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105
(901) 522-0300

ASSENT FORM: GENE THERAPY FOR RECURRENT PEDIATRIC BRAIN TUMORS

You have a brain tumor that has not gotten better with standard treatment. We would like you to be in a study of a very new type of treatment called gene therapy. For this treatment, a special virus will be used to carry a "target gene" (a piece of DNA) into the tumor cells. Tumor cells with the target gene should die when they come in contact with an anti-virus drug called "ganciclovir." Because the gene should only go into the tumor cells, normal brain cells should not be hurt. This treatment has been done in some adults with brain tumors, but this is the first study in children.

We will need to do several tests before the treatment is given. We need to check for tumor cells in the spinal fluid (spinal tap) and test for HIV (the virus that causes AIDS). If these tests are positive, gene therapy can't be used. Also, pregnant patients can't have this treatment.

TREATMENT:

An operation will be done to put the special gene into the tumor cells. While you are asleep (general anesthesia), the neurosurgeon will guide a needle to the tumor through a small hole drilled in the skull. The cells with the target gene will be injected into the tumor. This may be done in more than one place if the tumor is large. Seven days later you will begin to get the ganciclovir. It will be given by vein (IV) twice a day for 14 days. You will be in the hospital for the treatment. We will do MRI scans several times after treatment to see if the tumor is getting smaller. You will need to be seen often in the clinic after the treatment for scans and blood tests.

RISKS AND SIDE EFFECTS

The operation to put the target gene in the tumor can cause severe bleeding, worsening of strength or feeling, and problems talking and remembering things. There is also risk of infection. The neurosurgeon will talk to you about the chances of these bad effects.

The special virus that carries the gene has been used in many patients before and has not caused problems. There is a very small chance that the virus could cause infection, but we don't think this will happen. If the target gene gets into normal brain cells, those cells could be killed too. We do not think this will happen, but we can't be sure.

Gancyclovir has been used to treat viral infections in many people. Its main bad effect is low blood counts, which can lead to infection and bleeding. We will stop the drug if counts go too low.

Because this therapy is so new, there may be other bad effects that we do not yet know about.

Patient	Parent
Physician	Witness
Date	Time

In case of questions or emergencies in reference to this protocol, please contact:
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105
(901) 522-0300

POSSIBLE BENEFITS

We hope that this treatment can make the tumor smaller, but we do not know this. What we learn from this study may help other patients in the future.

ALTERNATIVE TREATMENTS

There are no standard treatments that are likely to help at this point. There may be other research treatments that could be used and your doctor will discuss this with you. You may also prefer not to have any more treatment.

STATEMENT OF UNDERSTANDING

Information about your treatment will be known only by the hospital staff who need to know. Doctors working with this gene therapy in other hospitals will also need to know about your treatment. We will not discuss it with anybody else without asking you first. You have the right to ask any questions you want and they will be answered. You have read this form, or had it read to you, and discussed it with your parents and the doctor (Dr. _____) caring for you. Before you signed this form, you had all your questions answered.

If you have any other questions, you can ask your doctor or other people caring for you at any time.

_____ _____
Patient Parent

_____ _____
Physician Witness

_____ _____
Date Time

In case of questions or emergencies in reference to this protocol, please contact:
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105
(901) 522-0300

BIOGRAPHICAL SKETCH

Give the following information for all new key personnel, consultants, and collaborators.
Copy this page for each person.

CVs

NAME	POSITION TITLE
KUN, Larry E.	Chairman, Radiation Oncology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Pennsylvania State University, University Park	B.S.	1966	
Jefferson Medical College, Philadelphia, PA	M.D.	1968	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individual who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1974 National Cancer Institute, NIH, Radiation Branch, Bethesda, MD (Clinical Associate in Radiotherapy)

1974 Rotterdam Radiotherapy Institute, Rotterdam, The Netherlands (Assistant Radiotherapist) (June-December)

1975-76 Assistant Professor of Radiology, University of Vermont, College of Medicine, Medical Center Hospital of Vermont, Burlington

1976-80 Assistant Professor of Radiation Oncology, Medical College of Wisconsin

1980-84 Associate Professor of Radiation Oncology, Medical College of Wisconsin, Milwaukee

1986 to present Professor and Director, Section of Radiation Oncology, University of Tennessee, College of Medicine, Memphis

1987 to present Professor of Pediatrics, University of Tennessee, College of Medicine, Memphis

1984 to present Chairman and Member, Department of Radiation Oncology, St. Jude Children's Research Hospital, Memphis, TN

MOST RECENT PUBLICATIONS

Horowitz ME, Kun LE, Mulhern RK, Kovnar EH, Sanford RA, Hockenberger BM, Greeson FL, Langston JW, Fairclough DL, Jenkins JJ III. Feasibility and efficacy of preirradiation chemotherapy for pediatric brain tumors. Neurosurgery 22:687-690, 1988.

Horowitz ME, Mulhern RK, Kun LE, Kovnar E, Sanford RA, Simmons J, Hayes FA, Kenkins JJ III. Brain tumors in the very young child. Postoperative chemotherapy in combined-modality treatment. Cancer 61:428-434, 1988.

Schewe KL, Reavis J, Kun LE, Cox JD. Total dose, fraction size, and tumor volume in the local control of Hodgkins disease. Int J Radiat Oncol Biol Phys 15:25-28, 1988.

Freeman CR, Krischer J, Sanford RA, Burger PC, Cohen M, Norris D, Kun L. Hyperfractionated radiotherapy in brainstem tumors: Results of a Pediatric Oncology Group study. Int J Radiat Oncol Biol Phys 15:311-318, 1988.

Kun LE, Kovnar EH, Sanford RA. Ependymomas in Children. Pediatric Neuroscience 14:57-63, 1988.

Stephenson JA, Mayland DM, Kun LE, Etcubanas E, Thompson EI, Gross CW. Malignant germ cell tumors of the head and neck in childhood. Laryngoscope 99:732-735, 1989.

Mulhern RK, Horowitz ME, Kovnar EH, Langston J, Sanford RA, Kun LE. Neurodevelopmental status of infants and young children treated for brain tumors with pre-irradiation chemotherapy. J Clin Oncol 7:1660-1666, 1989.

Pao WJ, Hustu HO, Douglass EC, Beckford NS, Kun LE. Pediatric nasopharyngeal carcinoma: long term follow-up of 29 patients. Int J Radiat. Oncol Biol Phys 17:299-305, 1989.

Halperin EC, Kun LE, Constine LS, Tarbell NJ. Pediatric Radiation Oncology. New York: Raven Press, 1989.

Kovnar EH, Kellie SJ, Horowitz ME, Sanford RA, Langston JW, Mulhern RK, Jenkins JJ, Douglass EC, Etcubanas EE, Fairclough DL, Kun LE. Preirradiation cisplatin and etoposide in the treatment of high-risk medulloblastoma and other malignant embryonal tumors of the central nervous system: A Phase II Study. J Clin Oncol 8:330-336, 1990.

Tobin RL, Fontanesi J, Kun LE, Fairclough DL, Hustu HO, Pao WJ, Douglass EC, Wilimas J, Kumar APM, Jenkins JJ. Wilms tumor: reduced-dose radiotherapy in advanced stage Wilms tumor with favorable histology. Int J Radiat Oncol Biol Phys 19:867-871, 1990.

Crist WM, Kun LE. Common solid tumors of childhood. N Engl J Med 324:461-471, 1991.

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Castleberry RP, Kun LE, Shuster JJ, Altshuler G, Smith IE, Nitschke R, Wharam M, McWilliams N, Joshi V, Hayes FA. Radiotherapy improves the outlook for patients older than 1 year with Pediatric Oncology Group Stage C neuroblastoma. J Clin Oncol 9:789-795, 1991.

Krischer JP, Ragab AH, Kun L, Kim TH, Laurent JP, Boyett JM, Cornell CJ, Link M, Luthy AR, Camitta B. Nitrogen mustard, vincristine, procarbazine, and prednisone as adjuvant chemotherapy in the treatment of medulloblastoma. A Pediatric Oncology Group study. J Neurosurg 74:905-909, 1991.

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Kun LE, Moulder JE. General principles of radiation therapy. In Pizzo PA, Poplack DG, eds. Principles and Practice of Pediatric Oncology. Philadelphia: JB Lippincott Co (in press), 1992.

CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE *(Mo., Day, Yr.)*
SANFORD, Robert A.	Consultant, Pediatric Neurosurgery	June 23, 1941

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Hendrix College, Conway, AR	B.A.	1963	
University of Arkansas, School of Medicine, Little Rock, AR	M.D.	1967	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1973-1975 Chief of Neurosurgery, U.S. Army Hospital, Camp Kue, Okinawa, Japan
1975-1979 Assistant Professor of Neurosurgery, University of Mississippi Medical Center, Jackson, MS
1979-1984 Associate Professor of Neurosurgery, University of Mississippi Medical Center, Jackson, MS
1975-1984 Chief of Pediatric Neurosurgical Service, University of Mississippi Medical Center, Jackson, MS
1985 to present Clinical Professor, University of Tennessee Medical Center, Memphis, TN
1985 to present Chief of Pediatric Neurosurgery, LeBonheur Children's Medical Center, Memphis, TN
1984 to present Pediatric Neurosurgery, St. Jude Children's Research Hospital, Memphis, TN

PUBLICATIONS

McNair JL, Clower BR, Sanford RA. Effect of reserpine pretreatment of myocardial damage associated with stimulated intracranial hemorrhage in mice. Eur J Pharmacol 9:1-6, 1970.

Smith RR, Haining J, Sanford RA. Effect of anesthesia and hypercardiac on the subcortical local blood flow in the monkey brain. Surg Forum 2:420-421, 1971.

Patrick BS, Sanford RA. Management of pain of malignancy: III: Neurectomy and rhizotomy. J Miss St Med Assoc 18:276-278, 1977.

Patrick B, Gatapon G, Sanford RA. Management of pain of malignancy. J Miss St Med Assoc 18:137-140, 1977.

Patrick B, Gatapon G, Sanford RA. Management of pain of malignancy. J Miss St Med Assoc 18:230-232, 1977.

Sanford RA, Patrick B. Management of pain of malignancy: cordotomy. J Miss St Med Assoc 19:46-48, 1978.

Sanford RA, Smith RR. Intraventricular tobramycin in the management of intraventricular infections of infancy in childhood. Surg Forum 31:465-467, 1980.

Holla SP, Smith RR, Sanford RA. Bilateral traumatic facial paralysis. Neurosurgery 6:290-292, 1980.

Sanford RA, Bebin J, Smith RW. Pencil gliomas of the aqueduct of Sylvius. J Neurosurg 56:690-696, 1982.

Sanford RA, Romfh JH. Closure of large traumatic lumbosacral defects. Neurosurgery 11:234-238, 1982.

Sanford RA. Hydrocephalus and shunt malfunction. J Miss St Med Assoc 24:35-38, 1983.

Sanford RA, Laurent JP. Intraventricular tumors in children. Cancer 56:1795-1799, 1985.

Sanford RA, Smith RR. Hemangioblastoma of the cervico medullary junction. J Neurosurg 64:317-321, 1986.

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Langford HG, Sanford RA, Smith R, Currier R, Johnson W, Klein R, Bagget R. Neurogenic hypertension in man. J Hypertension 5:S467-469, 1987.

Freeman CR, Krischer J, Sanford RA, Burger PC, Cohen M, Norris D: Hyperfractioned radiotherapy in brain stem tumors, Results of a Pediatric Oncology Group study. Int J Oncol Biol Phys 15:311-318, 1988.

Horowitz ME, Mulhern RK, Kun LE, Kovnar E, Sanford RA, Simmons J, Hayes FA, Jenkins JJ. Brain tumors in the very young child: postoperative chemotherapy in combined-modality treatment. Cancer 61:428-434, 1988.

Horowitz ME, Kun LE, Mulhern RK, Kovnar EH, Sanford RA, Hockenberger PA, Greeson FL, Langston JW, Fairclough DL, Jenkins JJ. Feasibility and efficacy of preirradiation chemotherapy for pediatric brain tumors. Neurosurgery 22:687-690, 1988.

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Mulhern RK, Horowitz ME, Kovnar EH, Langston J, Sanford RA, Kun LE. Neurodevelopmental status of infants and young children treated for brain tumors with pre-irradiation chemotherapy. J Clin Oncol 7(11):1660-1666, 1989.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
BRENNER, Malcolm Keith	Member, Hematology/Oncology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Cambridge	MA,MB,BChir	1975	Medicine
Membership, Royal College of Physicians	MRCP	1977	Medicine
University of Cambridge	PhD	1981	Immunology
Membership, Royal College of Pathologists	MRCPath	1988	Haematology
Fellowship, Royal College of Physicians	FRCP	1990	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1975 House Physician to Professor M.D. Milne, Westminster Hospital, London, U.K.
1976 Senior House Officer to Professor J.P. Shillingford, Hammersmith Hospital, and Royal Postgraduate Medical School, London
1977 Senior House Officer to Professor M. Turner Warwick and Dr. P. Cole, Brompton Hospital, London
1977-78 Medical Registrar to Professor A.F. Lant and Dr. L.W. Loughridge, Westminster Hospital, London, U.K.
1978-81 Medical Research Council Training (MRC) Fellow, Division of Immunology, Department of Pathology, Cambridge University, U.K.
1981-83 MRC Clinical Scientist, Honorary Senior Registrar, Northwick Park Hospital, and Clinical Research Centre, London
1983-85 Lecturer in Haematology, Royal Free Hospital and Hospital for Sick Children, Great Ormond Street, London
1985-89 Senior Lecturer, Honorary Consultant Haematologist (Deputy Director, BMT Program) Royal Free Hospital, London, U.K.
1990 to present Professor, Departments of Pediatrics and Medicine, University of Tennessee, Memphis, Tennessee
1990 to present Member, Hematology/Oncology, and Director, Bone Marrow Transplant Program, St. Jude Children's Research Hospital, Memphis, Tennessee

SELECTED PUBLICATIONS (of 137 total)

Wimperis JZ, Brenner MK, Prentice HG, Reittie JE, Patterson J, Karayiannis P, Griffiths PD, Hoffbrand AV. Transfer of a functioning humoral immune system in transplantation of T lymphocyte depleted bone marrow. Lancet i:339-343, 1986.

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Cordingley FT, Bianchi A, Hoffbrand AV, Reittie JE, Heslop HE, Vyakarnam A, Turner M, Meager A, Brenner MK. Tumour necrosis factor as an autocrine tumour growth factor for chronic B-cell malignancies. Lancet i:1969-1971, 1988.

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

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CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

9309-056 Das Gupta, Tapas K. and Cohen, Edward P.; University of Illinois at Chicago; Chicago, Illinois; *Immunization of Malignant Melanoma Patients with Interleukin 2-Secreting Melanoma Cells Expressing Defined Allogeneic Histocompatibility Antigens.*

Date of RAC Approval: 9/10/1993
Date of NIH Approval: 4/19/1994

Technical Abstract

Abstract: A cell-line was established from the neoplastic cells of a patient with malignant melanoma. The cells were obtained during the natural course of the patient's treatment. The melanoma cells express defined MHC Class I histocompatibility determinants. The gene for human interleukin-2 (IL-2) was transduced into the cells with the aid of a replication-defective retrovirus. Integration of the gene into genomic DNA and its expression were established. The IL-2-secreting cell-line was found to be free of recombinant retro-viruses and infectious agents. It will be X-irradiated (5000 rads) and then used to immunize twelve informed patients with Stage IV malignant melanoma in a Phase I toxicity study.

Patients will become eligible for inclusion in this study only if they develop metastatic melanoma and have failed all standard forms of treatment. The IL-2-secreting melanoma cells will be injected into patients who differ in at least three of six alleles at the Class I locus. The patient's anti melanoma immune response to the injected cells will be determined by both *in vivo* and *in vitro* parameters. Background studies performed in inbred mice indicate that X-irriadated IL-2-secreting cells that express both melanoma-associated antigens and allogeneic Class I histocompatability antigens are more antigenic in terms of their capacity to induce an anti melanoma response than X-irradiated melanoma cells. Of significance for the future potential of this form of therapy, the period of survival of mice with established melanoma was significantly ($P < 0.001$) longer than that of untreated animals or animals treated with X-irradiated melanoma cells. There was no evidence that immunizations with the IL-2-secreting cells were toxic to the tumor-bearing recipients.

Non-Technical Abstract

The incidence of melanoma in the general population has risen at a rate exceeding that of any other malignancy except lung cancer in women. By the year 2000, 1 in 90 individuals is projected to develop this often fatal form of skin cancer. New forms of treatment are needed. For most types of cancer, stimulation of the patient's immune system is without apparent benefit. The tumor cells are resistant to the immune system. In melanoma, however, objective evidence indicates that the immune system, properly stimulated, can recognize and destroy the cancer cells. Although not likely to be curative, this form of therapy might become an adjunct to surgery, X-ray and chemotherapy, the conventional forms of treatment. This proposal describes a means of modifying characterized human melanoma cells maintained in the laboratory. The modifications are expected to increase their capacity to stimulate an anti melanoma immune response in melanoma patients. The modifications are carried-out by infecting the cells with a defective virus that carries a gene for interleukin-2, a natural hormone of the immune system. The infected cells incorporate the gene and secrete the hormone. Before injecting patients, the modified cells will be subjected to high doses of X-irradiation (5000 rads) to ensure that they cannot grow in the patient. Extensive testing will be performed beforehand to be certain that the cells do not carry bacteria or viruses that might be harmful. The period of survival of mice with melanoma treated with in an analogous manner was prolonged relative to that of untreated mice, although tumor growth recurred and the animals eventually died of their disease.

APPENDIX IX

Clinical Protocol

Treatment Schema

—

Admit the morning of administration of interleukin-2 (IL-2)-producing allogeneic melanoma cells (UISO-Mel-4-IL-2)

—

5×10^8 UISO-Mel-4-IL-2 cells in 5 ml of PBS injected intramuscularly

Simultaneously, two intradermal injections of 2×10^7 UISO-Mel-4-IL-2 cells in 0.1 ml of PBS at a distance of 3 cm from the therapeutic injection site.

—

Discharge patient 23 hours after immunization.

Repeat injections every three weeks for four immunizations

—

After first course, evaluate biweekly for laboratory evidence of an anti melanoma response; clinical status

—

Failure to develop anti melanoma immunity and/or anti melanoma disease progression ---> off study

—

Evidence of anti melanoma immunity and or tumor regression and the patient will receive four courses of injections over a period of one year.

1.0 Objective

To determine the toxicity of immunization of Stage IV melanoma patients with an IL-2-secreting, X-irradiated melanoma cell-line expressing defined allogeneic MHC-specified determinants. The following will be evaluated:

1.1 Toxicity of immunization.

1.2 Evidence for induction of humoral and cellular immunity to autologous melanoma.

1.3 Evidence for induction of humoral and cellular immunity to the allogeneic melanoma cell-line.

1.4 Evidence for anti melanoma immunity in the immunized patients.

2.0 Background

Two hundred years after human melanoma was first described, the incidence of melanoma is rising more rapidly than any other malignancy excepting lung cancer in women. Once extra regional metastasis has occurred, the two year survival rate is less than 5 percent (1). Our understanding of the management of advanced melanoma is insufficient and new treatment options are required. Such studies will help in the development of new strategies that are capable of defining new forms of therapy.

2.1 Surgical Therapy: Surgical excision is recommended for primary cutaneous melanoma. The extent of excision usually depends on the measured thickness (depth) of the cutaneous lesion. In general, smaller lesions (<0.76 mm) have an excellent prognosis; however, the chance of cure diminishes as the thickness increases. In deep-level melanoma (>3.0 mm), even in the absence of obvious spread, the incidence of metastatic disease in the regional nodes is high, resulting in a lower cure rate and shorter periods of disease-free survival. Despite the progress that has been made in implementing strategies for disease control based on the recognition of early primary melanomas, the mortality rates have risen because of melanoma's burgeoning incidence. This increase will translate to 6,700 deaths in the United States this year. The main reason for this increase in the death rate is the fact that the prognosis is grave after melanoma metastasizes extra regionally.

2.2 Chemotherapy: Treatment of advanced melanoma with chemotherapy is largely based on the use of dacarbazine--still the drug of choice, with a response rate of about 15 to 18% (2). Studies have produced conflicting results regarding potentially superior combinations of chemotherapy (3). Ahmann *et al* (4) recently argued that the survival rate beyond five years of 2% (in 593 patients with metastatic melanoma who were treated with systemic chemotherapy) was not clearly linked to objective responses; they reasoned that it was related temporally but not causally to the institution of treatment. Thus, current chemotherapy regimens used to treat metastatic melanoma are at best palliative and even then are only temporary and actually palliative for a small percentage of patients (5).

The mechanisms of action of tamoxifen, either singly or in combination with other chemotherapeutic agents, is still unclear. However, Cocconi *et al*(6) have concluded that dacarbazine and tamoxifen should now be considered standard therapy for patients with metastatic melanoma. Further, they suggest that tamoxifen, a mixed estrogen agonist and antagonist, modulates the cytotoxic effects of chemotherapy through an estrogen-dependent mechanism.

2.3 Immunotherapy of Melanoma: Metastatic melanoma has been a major target for tumor immunotherapy because of the well documented occurrence of spontaneous regression, the demonstrated detection of melanoma associated antigens (MAA) both in patients and in experimental animals, the ready availability of tumor tissue, and the lack of routinely effective therapeutic options. Early trials focused on active non-specific immunostimulation with microorganisms or their constituents (7). With the exception of intralesional BCG therapy for regional intradermal metastases, there was little success and interest waned. Advances in basic immunobiology and molecular biology have rekindled activity, and several conceptually appealing approaches have evolved.

2.4 Immunotherapy Studies: Immunotherapy for the treatment of malignant melanoma has been attempted previously. Recent efforts are summarized in the following Table.

Table 1
Treatment of Advanced Melanoma Patients with Vaccine (1982-1992)

Author	Year	Vaccine Preparation	# of Pts.	Results (Response)
Weisenburger *et al.*[8]	1982	Allogeneic tumor cells, intralymphatic	34	Response in 9/34
Cassel *et al.*[9]	1983	Allogeneic oncolysates	32	Increased disease-free interval and survival
Harris *et al.*[10]	1983	Allogeneic tumor extract, CFA	16	Response in 1/16
Calkins *et al.*[11]	1984	Allogeneic tumor cells, BCG, intralymphatic	44	No Response
Humphrey *et al.*[12]	1984	Allogeneic tumor homogenate	84	Increased disease-free interval and survival
Morton *et al.*[13]	1986	Allogeneic tumor cells, BCG	140	No effect
Tallberg *et al.*[14]	1986	Autologous tumor extract, polymerized, PPD	6	Increased disease-free interval and survival
Hersey *et al.*[15]	1987	Allogeneic viral oncolysate	80	Increased disease-free interval and survival
Plager *et al.*[16]	1987	Allogeneic viral oncolysate	24	No effect
Bystryn *et al.*[17]	1988	Allogeneic tumor cell, soluble antigen, alum	13	Response in 1/13
Berd and Mastrangelo[18]	1988	Autologous tumor cells, with cyclophosphamide	33	Response in 4/33
Slingluff *et al.*[19]	1988	Allogeneic tumor cells, BCG	1080	Slightly increased survival

Mitchell *et al.*[20]	1988	Allogeneic tumor homogenate, Detox	42	Response in 9/42
Wallack *et al.*[21]	1989	Allogeneic viral oncolysate	39	Increased disease-free interval
Morton *et al.*[22]	1989	Allogeneic tumor cells, BCG	98	Response in 4/15
Wiseman *et al.*[23]	1989	Autologous tumor cells, intralymphatic	32	Response in 3/32
Livingston *et al.*[24]	1985	Autologous VSV oncolysate	13	No response to Class 1 or Class 2 melanoma antigens
Livingston *et al.*[25,26]	1987/89	GM2 ganglioside,	24	Serological response to GM2 in 17/24

The data presented show that, despite various methods used in the past to vaccinate individual patients, therapeutic efficacy was significant in only a minority of patients. However, more recent methods are yielding improved results. Therefore, it is logical to pursue newer lines of investigation which use the improvements in our conceptual knowledge of the immune system to optimize the methods of immunotherapy for metastatic melanoma.

With the development of antigen-specific monoclonal antibodies, a number of proteins, glycoproteins, and glycopeptides were identified as cell-surface constituents of human melanoma and candidates for antibody-mediated immunotherapy. Prominent among them are the gangliosides GM2, GD2, and GD3. When administered with BCG as adjuvant therapy, after pretreatment with low dose cyclophosphamide (to counteract suppresser activity) in patients with melanoma, vaccines containing purified GM2 elicited production of GM2 antibodies (25,26).

The discovery of IL-2 (27) enabled the multi-log *in vitro* expansion of the numbers of the patients' own lymphocytes. In a significant proportion of patients, the re infusion of a large number of autologous lymphocytes (LAK cells) induced tumor regression. Rosenberg (28-30) initiated a clinical trial of LAK cells plus bolus IL-2 (to maintain LAK activity *in vivo*) in patients with a wide variety of tumors. Melanoma was among the most responsive. A recent report listed four complete and six partial regressions (21%) in a series of 48 evaluable patients (31). More recently, Rosenberg has recovered lymphocytes from tumors (TIL cells). Re-infusion of TIL cells led to CR and PR in approximately 35% of patients (32).

2.5: IL-2 is an immune mediator with a variety of effects on the function of the immune system. The effects have been described both in experimental animals and humans (33). Injection of IL-2 can mediate antitumor immune responses against established tumors in patients with a variety of neoplastic diseases, notably malignant melanoma and renal cell cancer. IL-2 is essential for the development of "helper" function; it mediates expansion of the number of immunoreactive cells bearing IL-2 receptors. Established tumor cells are selectively killed following IL-2 administration (non malignant cells remain unaffected). This supports the conclusion that structural differences between neoplastic and non-neoplastic cells are present; they can form the basis of a rational approach toward cancer immunotherapy.

Why potentially antigenic tumors develop in immunocompetent individuals is not clear. Possibly, cells escape detection by the immune system even though they express determinants that are potential "targets" for recognition and destruction. Cytotoxic T lymphocytes are capable of recognizing an extraordinarily wide array of relatively small peptides derived from larger macromolecules in the context of membrane-associated structures specified by the Class I major histocompatability locus (MHC). Therefore, the array of potential neoantigens is quite large and almost certainly includes neoantigens associated with primary and advanced neoplasms. Several lines of indirect evidence support the idea that progressively growing tumors remain unrecognized and do not undergo appropriate antigen processing. In inbred mice, a strong anti tumor immune response follows immunization of syngeneic recipients with tumor cells hybridized with cells expressing allogeneic MHC (34). In an analogous manner, tumor cells modified by transfection and expression of genes specifying allogeneic antigens exhibit immunity toward nontransfected, syngeneic tumor cells (35-37). Thus, the co-expression of foreign and weakly antigenic tumor-associated determinants provides a vehicle for recognition, antigen processing, and generation of an anti tumor immune response. At the same time, the expression of allogeneic MHC antigens protects the tumor-bearing recipient from the malignant proliferation of the tumor cell vaccine itself, a possible danger if using viable tumor cell "vaccines" that are syngeneic with the host. Further evidence that the co-expression of foreign antigens along with tumor-associated determinants augments immune recognition is provided by the beneficial effects of immunizing experimental animals with tumor cells that have been infected with nonpathogenic viruses (9) or with viral constructs expressing tumor-associated determinants. Tumor-bearing animals immunized with such immunogens develop anti tumor immune responses that are capable of prolonging survival under circumstances in which the unmodified tumor cells themselves are not antigenic.

Other approaches have been taken to modify tumor cells in order to increase the cells' antigenicity. In 1987, DNA from a highly malignant murine leukemia cell-line was transfected into a mouse fibroblast cell-line expressing defined allogeneic MHC antigens (35). Like any foreign graft, the transfected cells were rejected by mice syngeneic with the tumor. Mice rejecting the transfected foreign cells exhibited strong anti tumor immune responses and survived longer following tumor challenge than non immunized mice. Tumor cells were killed by host immune mechanisms when the animals were challenged by injections of viable leukemia cells. Subsequently, DNA from mouse melanoma cells was transfected into fibroblasts expressing allogeneic MHC antigens

(37,38). Colonies of transfected cells expressing MAA were identified and used to immunize mice syngeneic with the tumor. Following immunization, the animals exhibited evidence of cellular immunity toward the melanoma and survived significantly ($P < .001$) longer following challenge than non immunized mice or mice in various control groups (37). Thus, transfecting genes that specify melanoma-associated determinants into cells that express allogeneic histocompatability antigens generated a cellular immunogen capable of generating strong anti melanoma responses and prolonging survival.

Further evidence that growth of a potentially antigenic neoplasm results from a failure of immune recognition is provided by the results of experiments implant tumor cells with genes specifying defined cytokines. Genes for IL-2 were transduced into weakly antigenic tumor cells by means of replication-defective retroviruses (39,40). The IL-2-secreting tumor cells were rejected by mice syngeneic with the neoplasm under conditions in which unmodified cells grew progressively. Significantly, animals rejecting the IL-2-secreting malignant cells were at least partially immune to challenge with the unmodified cells. Secretion of IL-2 by the tumor cells by-passed the requirement for CD4+ T-helper cells in the induction of the anti tumor response; tumor immunity developed in the absence of this ordinarily essential subset of T lymphocytes. Thus, two components of the cellular vaccine augmented immune recognition of weakly antigenic neoplasms: the expression of tumor-associated determinants and secretion of IL-2 by tumor cells. Cells modified in this manner lost malignant growth properties in immune competent recipients. Animals rejecting the cells exhibited both *in vitro* and *in vivo* evidence of anti tumor immunity.

Does immunization with a cellular immunogen that combines IL-2 secretion and the expression of tumor associated determinants lead to an enhancement of the anti tumor immune response? Does the expression of allogeneic histocompatability antigens lead to an enhancement of anti tumor immunity response? Experiments performed in our laboratories indicate that inbred mice immunized with a construct that combined IL-2 secretion and co-expression of allogeneic antigens and melanoma associated antigens generated anti melanoma immune responses that were capable of prolonging the survival of mice with melanoma. In some instances, tumor-bearing animals rejected the neoplasm and survived indefinitely. The anti tumor immune response following immunization with a cellular immunogen that combined the immunogenic properties of IL-2-secretion and expression of allogeneic antigens and MAA exceeded those following immunization with immunogens that were deficient in one or more of these immunogenic properties. Constructs that did not express MAA but formed IL-2, or those that expressed MAA but did not secrete IL-2, were less immunogenic in both in vitro and in vivo tests of immunity. Immunization of mice syngeneic with the constructs led to weak anti tumor responses, pointing to the role of allogeneic antigens in the anti melanoma response. The rejection of the melanoma was mediated primarily by CD8+ T lymphocytes. If the construct secreted IL-2, CD4+ T-helper cells were not required for induction of the anti melanoma response.

In analogous studies, Fearon et al. (39) transfected the IL-2 gene into a murine tumor cell line, CT26. Like B16 melanoma, CT26 cells ordinarily grow without inhibition in syngeneic (BALB/c) recipient mice. The IL-2-secreting tumor cells lost their malignant growth properties and were rejected by BALB/c mice. Significantly, mice rejecting the modified cells were partially

resistant to injections of unmodified CT26 tumor cells. The resistance was mediated primarily by CD8+ cytotoxic T lymphocytes. The anti tumor immunity generated by the IL-2-secreting gene-modified tumor cells was specific, because other non-IL-2-secreting tumor cells grew without inhibition in mice rejecting the IL-2-secreting tumor. Injections of unmodified tumor cells inactivated by high dose X-irradiation were not immunogenic and did not provoke anti tumor immune responses capable of protecting the animals against growth of non irradiated cells. Thus, secretion of IL-2 by the malignant cells eliminated their malignant growth properties in syngeneic mice. Animals rejecting the IL-2-secreting tumor cells were at least partially resistant to challenge with unmodified tumor cells.

In analogous studies, Gansbacher et al. (40) transduced a weakly immunogenic mouse fibrosarcoma cell line with a retroviral vector carrying the gene for IL-2. As previously, secretion of IL-2 by the tumor cells abrogated the cells' tumorigenic properties and stimulated long-lasting T cell mediated immunity against challenge with the unmodified tumor cells. Russell et al. (41) obtained similar results in an analogous system. They also observed tumors developing in animals injected with the IL-2-secreting cells were also rejected. In some instances, tumors developed following injection of the gene-modified cells. These cells were IL-2 negative variants. Our laboratory has confirmed these data for IL-2-secreting B16 melanoma injected into syngeneic C57BL/6 mice. One hundred percent of mice injected with IL-2-secreting melanoma cells developed tumors.

Rosenberg et al. (42) introduced the gene for IL-2 into a mouse sarcoma cell line (MCA-105). The IL-2-secreting cells were rejected by mice syngeneic with MCA-205 cells. Mice subjected to sub lethal irradiation or depleted of T cells supported the growth of IL-2-secreting MCA-205 cells, suggesting that immune mechanisms were responsible for rejection of the modified cells. Similar results were obtained for an independently arising sarcoma cell line (MCA-102). In each instance, mice rejecting the IL-2-secreting cells developed partial immunity to the growth of non-IL-2-secreting, unmodified tumor cells. IL-2 secretion by highly malignant tumor cells led to an increase in the cells' immunogenicity, abrogated the cells' malignant growth properties, and stimulated T cell-mediated anti tumor responses.

Are the immunogenic properties of IL-2 secreting tumor cells sufficient to lead to the regression of established tumors? To investigate this question, we first established melanomas in C57BL/6 mice. Six days afterwards, we administered the first of three weekly injections with allogeneic IL-2-secreting cell-constructs expressing MAA. The results are presented in Manuscript 1; Appendix VII. They indicate that animals rejecting the IL-2-secreting cells survived significantly ($P < .001$) longer than untreated mice or mice treated according to the same protocol with X-irradiated B16 cells.

Berd and Mastrangelo (18) investigated the immunogenic properties of irradiated autologous tumor in melanoma patients. They found infiltration of T cells in existing tumor and objective clinical regression of metastatic melanoma (19). Mitchell et al. induced objective clinical responses in 109 melanoma patients by active immunotherapy with pooled allogeneic melanoma cell lysates administered along with an immunological adjuvant (Detox*) (20). The response was mediated primarily by cytotoxic T lymphocytes. Numerous other reports indicate the importance of T cells in the rejection of established

neoplasms. T cells isolated from lymph nodes, peripheral blood lymphocytes, or tumor infiltrating lymphocytes (TIL) reacted against melanoma in patients receiving various forms of immunotherapy. The specificity of the T cell response was directed against a common MAA.

2.7 Rationale: The objective is to determine if immunization of Stage IV malignant melanoma patients with an IL-2-secreting allogeneic melanoma cell-line is tolerated without evident toxicity. The use of established cell-lines by-passes the necessity of modifying autologous tumor cells for each patient. Growing autologous tumor cells in cell culture can be a technically difficult and is not always successful. The emergent cells may be a non representative subline. Our laboratories established a melanoma cell-line from a patient with malignant melanoma (see below for details). Recent evidence, both in our laboratory and in the laboratories of other investigators, indicates that IL-2 secretion by tumor cells increases the frequency of tumor specific cytotoxic precursors and cytotoxic T lymphocytes.

2.8 The melanoma cell-line: Characteristics of the melanoma cell-line (UISO-MEL-4) are presented in Table 5.

2.9 Clinical Protocol: This is a pilot study of immunization with allogeneic melanoma cells that have been modified to secrete IL-2. They will be injected into selected patients with Stage IV metastatic melanoma who differ at three of six alleles specified by the MHC Class I locus. The expected duration for the trial is 12-15 months. The safety of the procedure will be established during this period.

The melanoma cells were transduced with the retroviral vector pZipNeoSVIL-2. The IL-2-secreting cells will be irradiated (5,000 rads) and administered in a series of four immunizations. If a patient has a response, as defined in section 10.0, then additional immunizations will be administered for up to one year in duration or until the disease progresses. Six patients will receive a 5×10^8 cells intramuscularly + 2×10^7 cells intradermally (2 injections), and the remaining six will receive a one log higher number of cells (see section 7.1).

3.0 Criteria for Patient Eligibility

Patients must fulfill the following criteria to be eligible for admission to the study:

3.1 Histologic confirmation of metastatic malignant melanoma at the University of Illinois, Department of Pathology.

3.2 Stage IV disease that can not be controlled by conventional therapy.

3.3 Measurable or evaluable disease.

3.4 Karnofsky performance status $\geq$ 70% and life expectancy > four months (Appendix II).

3.5 Adequate hematologic function, with WBC $\geq 3000/mm^3$, absolute lymphocyte count $\geq 1000/mm^3$, hemoglobin ≥ 9, and platelets $\geq 100,000/mm^3$.

3.6 Adequate renal and hepatic function (serum creatinine $\leq$ 2.5 mg/dl and bilirubin $\leq$ 1.7 mg/dl).

3.7 At least four weeks since any prior radiation therapy, immunotherapy, or chemotherapy (six weeks for nitrosoureas), and full recovery from such treatment.

3.8 The ability to give written informed consent.

3.9 Age $\geq$ 18 years.

3.10 No history of other concurrent malignancies except basal cell carcinoma or carcinoma *in situ* of the cervix.

3.11 No systemic steroids for at least one week prior to treatment.

3.12 No intracranial or multiple hepatic metastases.

3.13 Availability of fresh or frozen autologous tumor suitable for lymphocyte proliferation studies.

4.0 Exclusion Criteria

The following conditions/situations will render patients ineligible for this study:

4.1 Serious intercurrent medical illness, to include significant cardiac disease, angina pectoris, or myocardial infarction within the previous six months.

4.2 Active infection, including urinary tract infection.

4.3 Active intracranial metastases. Previously resected intracranial disease and or previously irradiated intracranial metastases which have been stable for 4 weeks are eligible.

4.4 Multiple hepatic metastases (tumor replacing 50% or more of liver parenchyma).

4.5 Pregnant or lactating women.

4.6 Patients requiring systemic corticosteroids.

5.0 Concurrent Therapy

The following therapies are prohibited while patients are being treated on this protocol: radiation therapy, chemotherapy, corticosteroids (except as administered for life-threatening circumstances), or other immunizations.

6.0 Pretreatment Evaluation

6.1 History and physical examination, including documentation of all measurable disease.

6.2 Height, weight, and performance status.

6.3 Screening profile, BUN, and creatinine.

6.4 CBC, differential, and platelet count.

6.5 EKG

6.6 Chest X-ray and CT scans of the chest and abdomen.

6.7 Magnetic Resonance Image or CT scan of the brain.

6.8 Additional CT scans as indicated to evaluate disease

6.9 HLA typing.

6.10 Anergy Battery: 7-antigen skin testing with control using the Merieux Tine Test will be placed and read 40 to 54 hours after placement.

6.11 Skin Test: 10^7 washed and irradiated (5000 rads) UISO-MEL-4-IL-2 cells will be administered intradermally and the resultant induration at 40 to 54 hours will be measured.

6.12 Peripheral blood will be obtained for immune assay. These will be delivered to the laboratories of Dr. Cohen (Room 717, MSB; extension 6-9479).

6.12.1 Two (2) 10 cc Vaccutainer tubes without additive ("Tiger" top or Red top) will be obtained for pretreatment serum.

6.12.2 Four (4) 10 cc Vaccutainer tubes containing heparin (Green top) or 40 cc of heparinized blood will be obtained for lymphocyte proliferation assays.

7.0 Immunization Plan

7.1 Patients will receive at least four courses of immunization. The first six patients will receive a low number of injected cells. The subsequent six will receive the higher number. The immunizations will consist of irradiated (5000 rads) IL-2-secreting melanoma cells expressing defined MHC Class I-determinants.

Low Dose: 5×10^8 intramuscular (IM) and 2×10^7 (x2) intradermal (ID)
High Dose: 5×10^9 IM and 2×10^8 (x2) ID

7.2 Patients will be admitted to the University of Illinois Hospital the morning of the administration of the IL-2-secreting cells. Patients will remain in the hospital for 23 hours following each immunization.

7.3 A series of four courses of immunization will be administered intramuscularly and intradermally into either the thigh or the arm of the patient. The sites will vary with each immunization. A limb that has undergone a lymph node dissection will not be used for the injection site. One course of therapy will consist of four (4) immunizations administered every three weeks. Thus, the last of the four injections in a course will be administered at week 9.

If a patient develops a significant titer of anti melanoma antibody without evidence of disease progression or has a clinical response (section 10.0), four additional courses will be given over a period of one year.

Blood tests will be performed prior to each immunization (see section 8.0 and addenda).

7.4 If a patient develops a severe reaction to the vaccine, defined as grade 4 toxicity (see section 11.0), or deteriorating performance status necessitating hospitalization, subsequent immunizations will not be administered.

7.5 Criteria for removal from study:

7.5.1 Progression of disease (section 10.0). Appearance of small (< 2 cm diameter) superficial cutaneous or subcutaneous metastases prior to week 9 will not be considered disease progression.

7.5.2 Severe intercurrent illness.

7.5.3 Recurrence of any grade 3 toxicity.

7.5.4 Any grade 4 toxicity.

7.5.5 General or specific alteration in the patient's condition which would render further treatment unacceptable in the judgment of the principal investigator.

7.6 Formulation of immunogen: The established melanoma cell-line was transduced with the retrovirus pZipNeoSVIL-2 (Dr. Cohen). After primary selection (G418), the secretion and quantitation of human IL-2 for both X-irradiated (5000 rads) and non irradiated cells was confirmed. No cells subjected to X-irradiation survived. Aliquots of the suspension of IL-2-secreting allogeneic cells are stored in liquid nitrogen (Dr. Cohen). Immediately before immunization, vials containing approximately 10^9 cells will be thawed, washed, counted and subjected to 5000 rads X-irradiation. Afterwards, the cells will be delivered to the Surgical Oncology Clinic for intramuscular and intradermal injections into tumor-bearing melanoma patients by the principal investigator. The frozen cells will have been found to be free of recombinant helper virus, myoplasma, bacteria, fungi and other adventicious agents.

8.0 Evaluation during study.

8.1 Physical examination before each immunization.

8.2 CBC, platelet count, and differential before each immunization.

8.3 Liver function tests (including bilirubin alkaline phosphatase, SGOT and lactic dehydrogenase) and kidney profile (including serum electrolytes, creatinine and BUN) before each odd-numbered immunization.

8.4 Peripheral blood samples will be obtained for humoral and cellular immune assays before each immunization and at two weeks after the fourth immunization.

8.5 Tumor evaluation will be performed during week eleven of each course of immunization. The evaluation consists of a physical examination, CT scans of the chest and abdomen, and a repetition of any other studies performed before the first immunization that revealed evidence of disease.

8.6 Patients removed from study without disease progression must be have repeat tumor evaluations every eight weeks as described in section 8.5. In addition, the patients will undergo physical examination every 4 weeks after removal from study until progression of disease is documented.

9.0 Measurement of an Effect

9.1 Humoral Immune Response: The most direct method to measure an immune response to melanoma is detection of anti melanoma antibodies. Approximately one-third of patients with metastatic melanoma have antibodies that react with autologous tumor cells (43). Most of these auto antibodies are IgM but on occasional IgG antibodies are present. Several antigens recognized by the auto antibodies react with the acidic glycolipids GM2 and GD2, and the melanosomal glycoprotein gp 75 (43). The detection of IgG antibodies induced by immunization is also an indirect measurement of helper T cell responses. All patients will have peripheral blood sera obtained for serologic analysis of antibodies directed against both the IL-2-secreting cell-line (to include antibodies against GD2 and gp 75) and autologous tumor cells. In addition, as a control for a positive immune response, tests for the detection of antibodies directed toward allogeneic MHC Class I antigens expressed by the cell-line will be performed. IgM and IgG antibodies toward cell surface antigens expressed by UISO-MEL-4-IL-2 cells will be detected by quantitative immunofluorescence. The same approach will be used to detect the induction of antibodies toward autologous tumor.

9.2 Cellular Immune Response: MHC-restricted cellular cytotoxicity toward autologous tumor will be evaluated in the immunized patients. Although cellular immune responses are a critical measure of immune responses to melanoma, we realize the following limitations: a) autologous tumor cells are necessary to detect responses; b) autologous normal cells and MHC-matched allogeneic cells are necessary to evaluate the specificity of responses; c) evidence of cytotoxic or helper responses are not evident without *in vitro* stimulation by autologous tumor cells.

Mononuclear cells from peripheral blood will be purified by Ficoll-Hypaque gradient centrifugation. Cells will be cultured with autologous tumor cells, IL-2 (20-1000 units/ml), and autologous or allogeneic stimulator cells for 7-14 days. The following assays to detect the presence of cellular immunity will be performed:

Peripheral blood lymphocytes (PBL) will be cultured for 48 hours with autologous tumor cells, autologous normal cells, allogeneic tumor cells, or 100 nM OKT3 (a positive control). Cell proliferation will be measured by the uptake by ^{3}H-thymidine added to the cultures for the last six hours of the incubation period. These assays will be performed with PBL obtained from patients immediately before each immunization and 2 weeks after the fourth injections.

When autologous tumor cell lines are available, the cytotoxic response of PBL toward ^{51}Cr-labeled autologous tumor will be measured.

9.3 The immunocompetence of patients with metastatic disease may be diminished. To assess this possibility, delayed type hypersensitivity (DTH) reactions toward autologous tumor (5,000 rads) and against (5000 rads) UISO-MEL-4-IL-2 cells before and after the course of immunizations. It is recognized that generation of a DTH response to the cells does not indicate the presence of a cellular anti-melanoma response capable of controlling tumor growth *in vivo*.

9.4 As additional determinant of the induction of an immune response to the cellular immunogen the titer of cytokines in the peripheral blood following the administration of allogeneic vaccine will be measured. Although the appearance of cytokines in the serum is a non-specific indicator of immune stimulation, such monitors provide a valuable means of determining the host response to the immunization. They may be aid in determining which of the two doses of immunogen induces an immune response of greatest magnitude. Levels of TNF-alpha, IL-2, IL-6, and soluble IL-2 receptor will be measured by ELISA in serum samples obtained before and 24 hours following injection.

9.5 Interleukin-2 levels in patients' sera may also be increased as a result of IL-2 production by UISO-H-Mel-4-IL-2 cells. Sera obtained from patients 1, 2, 8, and 15 days after immunization will be assayed for IL-2 titers. A commercially available (Centocor) RIA assay kit is used for this purpose.

9.6 Tumor evaluation will be performed within 2 weeks of the first immunization and during weeks 10 or 11 of each course. Tumor evaluation after the completion of a course of immunization will include a physical exam and a CT scan of the chest and abdomen. Additional studies that revealed measurable disease before the first injections will be repeated following a course of immunization.

10.0 Criteria For A Therapeutic Response

10.1 Complete Response (CR): Disappearance of all clinical evidence of tumor by physical examination, X-ray, and CT scan for a minimum of four weeks.

10.2 Partial Response (PR): By physical examination or radiography (including CT scan and/or ultrasound), the patient maintains for a minimum of four weeks a decrease of over 50% in the sum of the perpendicular diameters of all measured lesions. No simultaneous increase in size of any lesion nor appearance of any new lesion may occur.

10.3 Minor Response (MR): 25-49% decrease in the summed products of diameters of measured lesions for a minimum of four weeks.

10.4 Stable Disease (SD): Less than 25% decrease or increase in tumor size for at least three months.

10.5 Progression (PROG): Greater than 50% increase in the sum of all measured lesions, or appearance of new lesions. Note that if there is the appearance of superficial cutaneous, subcutaneous, or soft tissue disease < 2 cm diameter, the patient may continue with immunizations at the discretion of the investigator.

10.6 Duration of Response: Measured from initiation of therapy until a 50% or greater increase from the smallest sum of all tumor measurements obtained best response (CR, PR, MR).

11.0 Criteria For Toxicity

Toxicity will be graded and classified according to Common Toxicity Criteria (clinical protocol Appendix I) by the following outline:

0 No toxicity

1 Mild toxicity, usually transient, requiring no special treatment and generally not interfering with usual daily activities.

2 Moderate toxicity ameliorated by simple maneuvers.

3 Severe toxicity which requires therapeutic intervention and interrupts usual activities. Hospitalization may or may not be required.

4 Life-threatening toxicity which requires hospitalization. A toxicity which causes a drug-related death will be called a 4F.

12.0 Adverse experience

To date, there have been minimal or no reported side effects from immunizations with allogeneic tumor cells (43). Patients have experienced induration and erythema at the immunization site. Anaphylaxis has not occurred. Growth of (non irradiated) allogeneic cellular immunogens has not been observed. (In this study, the cells are subjected to 5,000 rads before injection. There were no surviving cells *in vitro* following this dose of X-irradiation.) In the initial reported study of the administration of genetically altered human cells to patients, no additional adverse effects were reported (32).

Any adverse effects of treatment will be reported within 24 hours to the IRB of the UIC Cancer Center as well as the Office for Protection from Research Risks of the National Institutes of Health. A written report will follow within 10 days to the Office of Recombinant DNA Activities, Building 31, Room 4B11, Bethesda, MD, 20892.

13.0 Biostatistical considerations

13.1 The endpoint of this pilot study has been defined as clinical toxicity. As indicated in Section 7, six patients are to be injected at each dose level. If three patients develop grade III or IV toxicity (as defined by the Common Toxicity Criteria), then a maximum tolerated dose will be defined, and the study will be ended. All patients will be evaluated for immune response as described in section 9.0, although this evaluation will not be the only measurable endpoint.

13.2 Design and sample size

Twelve patients will be enrolled in this pilot study of allogeneic tumor cell immunization. Two dose levels of tumor cells will be used.

Low Dose: 5×10^8 intramuscular + 2×10^7 (2) intradermal

High Dose: 5×10^9 intramuscular + 2×10^8 (2) intradermal

13.3 The maximum tolerated dose will be defined as the dose of vaccine for which three or more patients have grade III or IV toxicity. The trial will be stopped at the determination of maximum tolerated dose or the completion of both dose levels. It is anticipated that a maximum tolerated dose may not be established in this study.

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APPENDIX III

Informed Consent

University of Illinois at Chicago
College of Medicine
Specialized Cancer Center
840 South Wood Street (M/C 820)
Chicago, Illinois 60612

Protocol Title: **Pilot Study of Toxicity of Immunization of Patients with Unresectable Melanoma with IL-2-Secreting Allogeneic Human Melanoma Cells**

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH

1. PARTICIPANT'S NAME

_____

I.D. NUMBER

_____

You are being asked to participate in a clinical research study. The doctors at the University of Illinois at Chicago (UIC) study the nature of disease and attempt to develop improved methods of diagnosis and treatment. This is called clinical research. In order to decide whether or not you should agree to be part of this research study, you should understand enough about its risks and benefits to make an informed judgment. This process is known as informed consent.

This consent form gives detailed information about the research study which the doctor will discuss with you. Once you understand the study, you will be asked to sign this form if you wish to participate. You will have a copy to keep as a record.

The title of the proposed research study is:

"Pilot Study of Toxicity of Immunization of Patients with Unresectable Melanoma with IL-2-Secreting Allogeneic Human Melanoma Cells"

2. PURPOSE OF THE RESEARCH STUDY

Treatment of patients with metastatic melanoma has been disappointing. In a very small number of patients, the cancer has disappeared without treatment. This suggests that an individual patient's immune system might be stimulated in such a way that makes it capable of fighting the cancer. For these reasons, new treatments that can increase an individual's immune response against his/her melanoma are being investigated. The purpose of this study is to evaluate the toxicity of injecting patients such as yourself with melanoma cells from another patient that have been modified genetically.

3. Description of Research.

This is a Phase I trial designed to determine if the injected material is toxic to patients with melanoma. The injected material consists of melanoma cells from another patient that have been modified genetically so that they secrete interleukin-2 (IL-2). IL-2 is a protein made by certain blood cells that can stimulate immunity. In earlier studies, cancer patients received large doses of IL-2. Some of these patients had reductions in the size of their tumors. Most of them experienced side effects. Sometimes these side effects were severe. Many of the side effects were a result of using very high doses of IL-2. This new technique will use much smaller doses of IL-2 to stimulate your immune system. We hope to determine whether or not this treatment is toxic, rather than how effective it is in treating your cancer.

Other options for the treatment of melanoma include chemotherapy, surgery, or radiation. You should feel free to discuss these options with one of your doctors. However, you are being offered this treatment because your tumor has not responded to standard treatment.

The tumor cells used for injection are stored melanoma cells from another patient with melanoma. These tumor cells have been induced by genetic modifications to produce IL-2. The altered cells now make IL-2. They will be X-rayed to prevent growth of the tumor cells in your body. This is the material you will receive, given into and under your skin in the arm or thigh.

Your injections will be administered in the Surgical Oncology Clinic of the University of Illinois Hospital. You will receive a total of four series of injections. For the first four course of injection, you will be admitted to the hospital for 23 hours of observation. You will not be responsible for hospitalization charges related to this one day period of observation. Blood tests will be performed before each series of injections. For this, a sample of blood (one or two ounces) is drawn from your vein before and after the injection. The blood is used to assess the response of your immune cells to the injected cells. At various intervals for the first four weeks and then monthly, you will need to be seen by your doctor for examinations. X-rays and scans will be taken before and after the injections to see whether the size of your tumor has changed.

After the first four courses, further injections will depend upon how your tumor responds and what kind of side effects you have. If you experience severe side effects, the injections will be stopped. If your tumors get much bigger or if new large-sized tumors develop, the injections may also be stopped. We cannot assure you that further injections with additional genetically modified cells will be possible, even if your tumor responds to the prior injections.

The dose of the IL-2-producing tumor cells will be varied. The first six patients in this research study will be treated with a standard number of tumor cells. If they have no adverse side effects, the next group of patients will be given a higher number.

It is not yet possible to say how helpful the injections will be in shrinking your melanoma, or what the optimal dose of altered tumor cells should be. This is a new technique that is currently being studied. The purpose of this study is to determine the safety of injections with these altered melanoma cells.

4. RISKS, SIDE EFFECTS AND DISCOMFORTS TO THE PARTICIPANTS

PRIOR STUDIES.

In previous published studies with X-rayed tumor cells, the side effects have been redness and swelling at the site of injection. No one has had a serious allergic reaction to the immunizations.

A virus was used to incorporate the blueprint for IL-2 into the melanoma cells you will receive. The virus cannot grow on its own. A similar virus was used in a recent study at the National Cancer Institute. Only a few patients received the virus, but so far there have been no serious side effects. However, this is a very new technique and serious side effects may yet be seen. There is a remote possibility that the irradiated melanoma cells used for the immunization produce a virus not detected with our screening methods. Should a virus be reproduced there is a possibility that you may develop symptoms of a viral infection. Members of this virus family are known to cause lymphomas (a form of cancer) in rats and mice. The risk is much lower than might occur following chemotherapy and irradiation. Should you suffer a severe reaction to the immunizations, the treatments will be discontinued.

You will be informed of the progress of the research study. During the time that you participate, you will be informed of any new findings which might affect your willingness to continue.

If you are injured as a result of your participation in this research study, emergency care will be provided free of charge. Hospitalization, and out-patient care will be available to you by the Hospital and billed to you as part of your medical expenses. No money will be provided by the University of Illinois Hospital or as compensation for a research-related injury.

5. POTENTIAL BENEFITS

The use of genetically modified cells may be of benefit to patients in future protocols but this research will not be of immediate benefit to patients entering this trial. The major benefit will be to patients in the future who are participating in this study.

6. ALTERNATIVE PROCEDURES OR TREATMENTS. RIGHT OF WITHDRAWAL.

Participation in this study is totally optional. Should you decline to take-part, you will continue to receive standard treatments available to others with a disease similar to yours. The choice to enter or not to enter the study is yours. You are in a position to make a decision if you understand what the doctor has explained and what you have read about the research study and other possible forms of care. If you decide not to participate, the other choices are available to you without prejudice. If you begin the study, you will have a right to withdraw at any time. If you should withdraw, you will be offered other available care which suits your needs and medical conditions.

7. FINANCIAL COST

You are responsible for the costs of the physician visits, hospitalizations, laboratory and diagnostic tests not related to this study. There will be no charge for the immunizations, the blood tests performed to evaluate the immune response to the injected material and the hospitalization for the 23 hour period of observation proposed in the trial.

8. PRIVACY

Your research and hospital records are confidential. Your name or any other personally identifying information will not be used in reports or publications resulting form this study. The National Institutes of Health, the Food and Drug Administration (FDA), or other authorized agencies as well as hospital personnel may inspect your records. A qualified representative of the National Cancer Institute and the University of Illinois' Institutional Review Board may inspect the patient/study records. All efforts will be made to ensure your privacy but this cannot be absolutely guaranteed.

9. NEED FOR EFFECTIVE CONTRACEPTION.

Participation in this study requires you to practice effective contraception. Should contraception be interrupted, or if there is a suspicion of pregnancy, you further participation in the study may be interrupted at the sole discretion of the Principal Investigator.

10. PERMISSION FOR POSTMORTEM IN THE EVENT OF DEATH.

Since this is a new study that may be of benefit of other patients, so as to gain the most possible knowledge, in the event of death you agree that a postmortem may be performed.

11. TERMINATION OF STUDY

Your participation in this research study may be ended without your consent for the following reasons

1. If your tumor does not respond or gets substantially larger during treatment.

2. If the doctors treating you see side effects that they consider dangerous.

3. If you refuse to have treatment as recommended by your doctor or if you refuse to have the tests needed to determine whether the treatment is safe and effective.

4. Pregnancy or failure to practice effective contraception.

12. PRIOR REVIEW.

This research study was reviewed by the Institutional Review Board of the University of Illinois at Chicago (UIC), the University's Biosafety Committee, and the NIH Recombinant DNA Advisory Committee of the National Institutes of Health. The UIC Board is legally responsible for making sure that research with patients is appropriate and that the patient's rights and welfare are protected.

The physician in charge of this research study is Tapas K. Das Gupta, M.D., Ph.D., telephone number (312) 996-6666. If you need more information about the study before you decide to join, or at any other time, you can contact him. In the event you decide to participate, Dr. Das Gupta or one of his collaborating physicians (Dr. Edward Cohen, (312) 996-9479 or Dr. Jon Richards (3120 996-6666) should be called whenever there are side-effects from the research study. The nurse oncologist who will assist you in all non physician-related questions and will coordinate your therapy program is Mary Trippon, R.N., telephone number (312) 996-6913.

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH

STATEMENT OF PHYSICIAN OBTAINING INFORMED CONSENT

I have fully explained this research study to the patient or guardian _____. In my judgment, and the patient's, there was sufficient access to information, including risks and benefits, to make an informed decision.

PHYSICIAN'S SIGNATURE _____

PHYSICIAN'S NAME _____

DATE _____

PATIENT'S STATEMENT

I have read the description of the clinical research study or have had it translated into language that I understand. I have also discussed it with the doctor to my satisfaction. I understand that this study is to determine whether or not this treatment is toxic, rather than how effective it is. I, _____, understand that my (patient's) participation is voluntary. I know enough about the purpose, methods, risks, and benefits of the research study to judge that I (the patient) want to take part in it.

PATIENT NUMBER _____

PATIENT SIGNATURE _____

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo.,Day,Yr.)
Tapas K. Das Gupta, M.D., Ph.D.	Professor of Surgery Director, Cancer Ctr.	Feb. 22, 1932

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Calcutta, India	MBBS	1953	Medicine
London University, England	Ph.D.	1967	Anatomy

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

Resident Training:

R.G. Kar Medical School and Hospital, Calcutta, India, 7/53-6/55.
Mount Sinai Hospital, Chicago, Illinois, 7/55-6/59.
Memorial Hospital for Cancer and Allied Diseases, New York, New York, 7/60-3/65.

Junior Attending, Gastric and Mixed Tumor Services, Memorial Hospital for Cancer and Allied Diseases, New York, New York, 1965-1968.
Assistant Professor, Cornell University Medical College, 1967-1968.
Associate Professor, Department of Surgery, University of Illinois, Abraham Lincoln School of Medicine, 1968-1970.
Consultant, Surgical Service, West Side Veterans Administration Hospital, Chicago, Illinois, 1968-present.
Chairman, Section of Surgical Oncology, Department of Surgery, Cook County Hospital, 1968-present.
Professor of Surgery and Head, Division of Surgical Oncology, Department of Surgery, University of Illinois at Chicago, 1968-present.
Director, Specialized Center for Cancer Research and Education, University of Illinois at Chicago, 1987-present.

Certified, American Board of Surgery, 1960.
Fellow of the American College of Surgeons
F.D.A., Chairman, Advisory Committee, General and Plastic Surgery, Section of the Surgical and Rehabilitation Devices Panel, 1982 to present.
National Institutes of Health Reviewers Reserve, 1988 - 1992.
National Institutes of Health, Chairman, Experimental Therapeutics Study Section, 1983 to 1988.
University of Illinois at Chicago, College of Medicine, Distinguished Professor Award, 1993

BIBLIOGRAPHY SHEET Tapas K. Das Gupta, M.D., Ph.D.

Dr. T.K. Das Gupta is the author of 228 publications.
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8. Harris JE, Bines S, Das Gupta TK: Therapy of disseminated malignant melanoma with recombinant alpha 2b-interferon and piroxicam: Clinical results with a report of an unusual response-associated feature (Vitiligo) and unusual toxicity (diffuse pulmonary interstitial fibrosis). Med & Ped Oncol, 1993 (Accepted for Publication).
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10. Levine EA, Trippon MJ, Das Gupta, TK: Preoperative Multimodality Treatment for Soft Tissue Sarcomas. Cancer, 1993 (Accepted for Publication).
11. Mehta RR, Graves JM, Hart GD, Shilkaitis A, Das Gupta TK. Growth and metastasis of human breast carcinoma with matrigel in athymic mice. Breast Cancer Research and Treatment 25:65-71, 1993.
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20. Stastny J.J., Das Gupta T.K. The Use of Daunomycin-Antibody Immunoconjugates in Managing Soft Tissue Sarcomas: Nude Mouse Xenograft Model. Cancer Res. 1993 (submitted).

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo.,Day,Yr.)
Cohen, Edward P.	Professor	September 29, 1932

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Washington University, St. Louis, MO	M.D.	1957	Medicine
U. of Chicago Hospitals, Chicago	Internship	1957-58	Medicine
National Inst. of Health, Bethesda, MD	Res. Assoc.	1958-60	Allergy/Infections
Univ. of Colorado, Boulder, CO	Res. Fellow	1960-62	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL DATA:

1964-1965 Assistant Professor, Department of Microbiology, University of Colorado.
1965-1967 Associate Professor, Institute of Microbiology, Rutgers, The State University of New Jersey.
1967-1968 Associate Professor, Departments of Microbiology and Medicine, Rutgers Medical School, New Brunswick, New Jersey.
1968-1969 Associate Professor, La Rabida-University of Chicago Insitute and the Department of Medicine, University of Chicago.
1969-1977 Associate Professor, La Rabida-University of Chicago Insitute and the Departments of Medicine and Microbiology, University of Chicago, and the Biological Sciences Collegiate Division, University of Chicago.
1971-1973 Assistant Dean-Coordinator for Basic Sciences Instruction in the Medical Curriculum, University of Chicago.
1972-1973 Chairman, Curriculum Committee, Pritzker School of Medicine, University of Chicago.
1977-1979 Professor, Department of Microbiology and La Rabida-University of Chicago Institute, the Departments of Medicine and Biological Sciences Collegiate Division, University of Chicago.
1979-1981 Dean, School of Basic Medical Sciences and Professor, Department of Microbiology and Immunology, University of Illinois College of Medicine.
1981-1983 Director, Office of Research Development, University of Illinois at Chicago.
1983-pres. Professor, Department of Microbiology and Immunology, University of Illinois at Chicago.
1988-89 Sabbatical, Imperial Cancer Research Laboratories, London, England, laboratory of Joyce Taylor-Papadimitriou Medical Center.
1992-pres. Professor, Department of Medicine and Member, Specialized Cancer Center, University of Illinois at Chicago.

FELLOWSHIPS:

1961-1963 Special Postdoctoral Fellow, U.S., Public Health Service.
1963-1965 Research Career Development Awardee, U.S. Public Health Service.

BOARD CERTIFICATION:

Diplomate of the National Board of Medical Examiners.
Diplomate of the American Board of Allergy and Immunology.

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77. Kim TS, Collins MKL Cohen EP. Immunization of mice with interleukin-2-secreting allogeneic mouse fibroblasts expressing mulanoma-associated antigens stimulates anti melanoma immunity mediated primarily by $CD8^+$ T lymphocytes. Submitted.

78. Characterization of immunoselected recurrent melanoma in mice treated with IL-2-secreting allogeneic cells expressing melanoma associated antigens: The effect of interferon-r or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) on the expression of $H\text{-}2^b$ antigens by recurrent melanoma cells. Submitted.

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7. Kohler H, Cohen EP. The role of antigen and antibody in idiotype regulation and and TL modulation. In: The Handbook of Cancer Immunology. H. Waters, ed. Garland STPM Press New York, 1981, pp. 191-218.

ABSTRACTS:

(Total of 81 - 1964-1992)

NONREFERED ARTICLES:

(Total of 46 - 1973-1992)

9309-057 Wong-Staal, Flossie; Poeschla, Eric; and Looney, David; University of California, San Diego, California; *A Phase I Clinical Trial to Evaluate the Safety and Effects in HIV-1 Infected Humans of Autologous Lymphocytes Transduced with a Ribozyme that Cleaves HIV-1 RNA.*

Date of RAC Approval: 9-10-93
Date of NIH Approval: 10-25-94

Scientific Abstract

HIV-1 infection causes progressive depletion of $CD4^+$ T lymphocytes and, after a period of clinical latency averaging 7-12 years, the clinical syndrome of AIDS in the overwhelming majority of patients. The gravity of the expanding pandemic and lack of effective treatments continue to spur a search for innovative therapies. Ribozymes are potentially therapeutic RNA molecules that contain anti-sense sequences for specific recognition, and RNA-cleaving enzymatic activity (11-20). We have shown that human T-cell lines and primary peripheral blood human T cells transduced with a hairpin ribozyme that cleaves HIV-1 RNA in the 5' leader sequence are intracellularly immunized against challenge with cloned and uncloned HIV-1 isolates (7,8,9). Escape or resistant virus is not detected after long term culture. The transduced T cells persistently express the ribozyme and there are no apparent deleterious effects on cell proliferation or long-term viability (9). The ribozyme acts at two steps in the viral lifecycle: by cleaving both afferent viral RNA genomes (9) and efferent viral mRNA expressed from integrated provirus (7,8,9). In this study, we will evaluate the safety and efficacy of ribozyme gene therapy in four to six patients with HIV-1 infection by reinfusing autologous $CD4^+$ T cells that have been transduced *ex vivo* with a retroviral vector that expresses the HIV-1 leader sequence ribozyme. The *in vivo* kinetics and survival of ribozyme-transduced cells will be compared by limiting dilution PCR with those of a separate aliquot of cells transduced with a control vector (identical except for the ribozyme cassette). The level and persistence of ribozyme expression will also be assessed. The results will determine whether this ribozyme can protect $CD4^+$ T cells in patients with HIV infection and will aid design of future trials of larger scale T cell replacement therapy and trials of hematopoietic stem cell gene therapy for HIV disease.

Non-Technical Abstract

HIV infection causes progressive destruction of the human immune system and eventually AIDS (Acquired Immune Deficiency Syndrome) in the overwhelming majority of patients. No curative treatments for HIV or AIDS currently exist. T cells are the main targets of HIV in the body. When a specific gene for a molecule called a ribozyme is introduced in the laboratory into human T cells they are protected (in the test tube) against HIV infection. The ribozyme does not have apparent harmful effects on the T cells. The ribozyme acts at two steps in the lifecycle of HIV: by cutting the genes of the virus as it enters cells and by cutting the genes of the offspring of virus in infected cells. In this study, we will evaluate the safety and usefulness of ribozyme gene therapy in four to six patients with HIV infection. We will do so by introducing the ribozyme gene into each patient's T cells in the laboratory and then transferring the cells back into the body. We will then study whether the lifespan of these cells is prolonged by having the ribozyme gene and whether there are side effects of the therapy. The results will determine whether this ribozyme can safely protect T cells in patients with HIV infection and will aid design of future trials of gene therapy for HIV and AIDS.

RIBOZYME GENE THERAPY FOR HIV-1 INFECTION

A PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY AND EFFECTS IN HIV-1 INFECTED HUMANS OF AUTOLOGOUS LYMPHOCYTES TRANSDUCED WITH A RIBOZYME THAT CLEAVES HIV-1 RNA

Principal Investigator: Flossie Wong-Staal, Ph.D.

Co-Principal Investigators: Eric Poeschla, M.D.
David Looney, M.D.

Investigators: Mang Yu, Ph.D.
Mark Leavitt, Ph.D.
Osamu Yamada, Ph.D.
Jiing-Kuan Yee, Ph.D.

University of California - San Diego

Submitted to the Recombinant DNA Advisory Committee on July 14, 1993

RIBOZYME GENE THERAPY FOR HIV-1 INFECTION

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RIBOZYME GENE THERAPY FOR HIV-1 INFECTION

AN OPEN-LABEL, CONTROLLED, PHASE 1 CLINICAL TRIAL TO EVALUATE IN HIV-1 INFECTED HUMANS THE SAFETY AND EFFECTS OF AUTOLOGOUS LYMPHOCYTES TRANSDUCED WITH A RIBOZYME THAT CLEAVES HIV-1 RNA.

Protocol

1. INTRODUCTION

1.1 Project Objectives

The principal objectives of this phase one, controlled trial of somatic cell gene transfer into HIV-1 infected humans are:

A) to determine the safety of infusing autologous lymphocytes that have been transduced *ex vivo* with a retroviral vector bearing an HIV-1 leader sequence hairpin ribozyme.

B) to compare *in vivo* in each patient the kinetics and survival of ribozyme-transduced cells with a separate aliquot of cells transduced with a control vector (identical except for the ribozyme cassette).

C) to determine the *in vivo* expression of the ribozyme in transduced lymphocytes.

D) to establish whether host immune responses directed against the transduced cells will occur *in vivo*.

E) to make preliminary observations on the effects of gene therapy with an anti-HIV-1 ribozyme on *in vivo* viral mRNA expression, viral burden and $CD4^+$ lymphocyte levels.

1.2 Background & Rationale for Gene Therapy of HIV Infection

A. The Nature and Magnitude of the Problem. HIV-1 infection causes progressive depletion of $CD4^+$ T lymphocytes and, after a period of clinical latency averaging 7-12 years, the clinical syndrome of AIDS in the overwhelming majority of patients. Twelve years after the first description of AIDS, the global pandemic continues to grow. Despite

unprecedented progress in understanding the causative agent at the molecular level, and in palliating the myriad complications of AIDS, HIV disease remains refractory to treatment. In 1993, death within a few years is a virtual certainty for patients with CDC-defined AIDS. Epidemiological considerations are also compelling: WHO projects that about 40 million people will have HIV infection worldwide by the year 2000 (3). More inclusive projections that take account of under-reporting suggest that the world at the turn of the century may harbor as many as 100 million people infected with HIV (4,5). Current WHO estimates for cumulative HIV infections since the start of the pandemic total over thirteen million and include the following subtotals: over 1.5 million in North America and Europe, 1.5 million in Latin America and the Caribbean, more than 8 million in sub-Saharan Africa, 75,000 in North Africa and the Middle East, and over 1.5 million in South and Southeast Asia (3,4).

More than 2 million of the world's HIV-infected people have developed AIDS; most of them have died (3). The social fabric of the African continent has been severely threatened by this disease--in some African cities, 30% adult infection rates are estimated and up to 80% of hospital beds are occupied by patients with AIDS (3,4). The explosive spread of HIV now being observed in South and Southeast Asia, the new epicenter, portends social calamity in those regions also. In addition to the horrific individual suffering, the world-wide economic losses stemming from this disease that strikes primarily in the most economically productive age groups are colossal and growing (4).

B. Rationale for Ribozyme Gene Therapy & Previous Data with the HIV-1 Leader Sequence Ribozyme. While the clear gravity of the pandemic compels consideration of innovative approache as well as streamlined Phase1 and 2 testing, it cannot of course alone justify experimental human trials. Why move ahead now with gene therapy in particular for HIV infection? At root, the HIV problem is a genetic one: infection with this retrovirus results in reverse transcription of the viral RNA and permanent integration of the resulting DNA into the genome of the infected cell (6). There the provirus becomes an inheritable feature of the cell and retains the potential for viral expression and production of progeny virus for the duration of the cell's lifetime. Patients with HIV-1 infection have become in effect, genetically mosaic for HIV. In this sense, AIDS is a genetic disease, albeit an acquired one.

It follows that effective treatment for HIV infection might engage the virus prior to integration, or in already infected cells, at the level of the integrated DNA provirus. No practicable methods now exist for selectively eliminating a particular chromosomally integrated DNA sequence from a population of human cells. As an alternative, therapies aimed at the RNA level (ribozymes, anti-sense RNA) could target and destroy incoming viral RNA genomes prior to their reverse transcription or destroy new viral RNA transcripts. Our previous data (see below and ref. 7,8,9 which are attached to this document) indicate that in transduced, G418-selected T cells *in vitro*, an HIV-1 leader sequence hairpin ribozyme does in fact inhibit HIV-1 infection at two steps in the viral lifecycle: by cleaving both incoming viral RNA genomes and viral RNA expressed from integrated provirus.

Most current gene therapy protocols use, as in this proposal, murine retroviral vectors to deliver therapeutic genes into target cells; this process (transduction) mimics the early events of retroviral infection (2,6,10,55). The crucial difference is that, unlike replication-competent retroviruses, the vector genome packaged within the viral coat contains no genes for viral proteins. The vector, LNL6, used in this study has been used in previous FDA-approved trials and is designed to have only long-terminal repeat sequences necessary for integration of the DNA intermediate into host cell chromosome and a packaging signal that allows packaging into viral structural proteins supplied by the packaging line in *trans* (2,10,37). We have inserted only a cassette containing a $tRNA^{val}$ promoter element to drive ribozyme expression and the ribozyme gene itself. Hence, genomic integration is the terminal step for the vector; it has no potential to make viral proteins in cells transduced with the packaged vector and therefore no opportunity to produce progeny virus.

Ribozymes are RNA molecules that contain anti-sense sequences for specific recognition, and RNA-cleaving enzymatic activity (11-20, 48). We have reported that a hairpin ribozyme derived from the negative strand satellite RNA of the tobacco ringspot virus [-sTRSV] and designed to cleave HIV-1 RNA in the 5' leader sequence (positions +111/112 from the cap site in HXB2) suppressed virus expression in HeLa cells co-transfected with proviral DNA from diverse HIV-1 strains, suggesting that the ribozyme was effective in blocking virus expression from pre-existing viral DNA (7). Subsequently, in experiments more directly analogous to gene therapy, human T-cell lines and normal primary peripheral blood T cells transduced with ribozyme-bearing retroviral vectors resisted challenge with diverse strains of HIV-1(9). No reverse

transcriptase activity or virus infectivity was detectable in the culture supernatants for up to 35 days (the duration of the experiments) which represents a more than 10^4-fold reduction in recovered virus. There was no detection of escape or resistant virus after long term culture. Identical cells transduced with a control vector (identical except for lacking the ribozyme cassette) were not protected. Furthermore, the ribozyme is effective against a broad spectrum of HIV isolates, including an uncloned clinical isolate (8,9). The latter more directly simulates the *in vivo* milieu where heterogenous viral quasispecies or "swarms" predominate.

Expression of ribozyme significantly decreased (50-100 fold) the efficiency of incoming virus to synthesize viral DNA. These results indicate that transfer and expression of the ribozyme gene interfered with both early and late events in the HIV replication cycle; this double-target "intracellular immunization" by a single agent makes the ribozyme a unique molecular genetic intervention for treatment of HIV infection. The transduced T cells persistently express the ribozyme (no diminution in expression has been seen in over 6 months) and, equally important, there are no apparent deleterious effects on cell proliferation (assessed by thymidine uptake) or long-term viability (9). The three publications (ref. 7,8,9) detailing these previous results are attached (following REFERENCES).

We have generated a retroviral particle-producing cell line, PAMJT, by the following methods: the ecotropic packaging cell line ψ2 was transfected by the calcium-phosphate method with a retroviral vector carrying the ribozyme driven by the human $tRNA^{val}$ promoter cloned in a widely used (2,10,21-25,37) vector for gene therapy, pLNL6. The transfections were performed in DMEM supplemented with HAT (hypoxanthine, 13μg/ml, thymidine 3.9 μg/ml and aminopterin 18 ng/ml) and 10% fetal bovine serum (FBS). The culture supernatants were filtered through 0.45 μm pore size filter to remove cell debris and then used to infect the amphotropic packaging line PA317 in the presence of polybrene (final concentration 4 μg/ml). The infected PA317 cells were grown in HAT medium containing G418 (400 μg/ml), changing the medium every three days. G418 resistant colonies were scored and picked and expanded. A high titer clone, PAMJT, (10^5 CFU/ml) was identified by the standard method and frozen. Higher titre producing clones are in preparation. To produce retroviral particles (MJT) for the transduction protocol described below, the frozen PAMJT will be thawed and selected in DMEM with HAT and G418 and the cells will be expanded in large quantity so that 500 ml of supernatant containing MJT retroviral particles will be obtained. The supernatant so

produced to date has been tested by the standard marker rescue procedure and contains no helper virus. We will test for helper virus again (see section 6 of protocol and Points to Consider for methods) and the helper virus-free supernatant will be aliquoted and frozen at -80°C.

C. Justification for Proceeding to a Human Trial & Discussion of Animal Models. We are proceeding to a Phase I human trial now for numerous reasons that include: 1) the pressing need for therapeutic advances, 2) the fact that pharmacological therapy with presently available primary anti-retroviral agents such as nucleoside reverse-transcriptase inhibitors (AZT, ddI, ddC) has proved toxic and of very limited efficacy (27), 3) the unique simplicity and long term benefits that gene therapy potentially offers for HIV treatment, 4) the availability of a retroviral vector and packaging line previously tested to be safe in humans (2,24,25,37) and 5) the lack of a good animal model for HIV-1 pathogenesis (and hence for *in vivo* testing of therapies).

In particular, the lack of an animal model for AIDS, despite an extraordinarily wide-ranging and intensive search for one, has led to a broad consensus that many questions crucial to experimental HIV vaccine and treatment development must be answered directly in human trials (26). No available animal model of HIV-1 infection adequately mimics the infection and pathogenesis process in humans. The only animals well-documented to be susceptible to HIV-1 infection are the chimpanzee and the gibbon ape. However, these animals develop neither disease nor immunological correlates of disease such as CD4-depletion. In addition, chimpanzees and gibbons are endangered species. (26-36)

While persistent experimental HIV-2 infection of macaques has been achieved, the animals again do not commonly develop syndromes comparable with AIDS (26). Infection of Asian macaques with SIV results in an immune-deficiency syndrome (29). However, these animals do not develop immune responses analogous to human infected with HIV-1 and there are important differences such as the invariant V3 region of the envelope protein in SIV (29,31). Moreover, because of the marked divergence of HIV-2 and SIV from HIV-1, a ribozyme recognizing a totally different target sequence would have to be evaluated and this would not be truly indicative of either efficacy or risk of administration of an HIV-1 ribozyme.

The SCID-Hu mouse model has several deficiencies and resembles primary HIV infection rather than AIDS; these mice are also devoid of human macrophages (33,34,35). Nevertheless, in studies that are currently ongoing, we are studying the acute and subacute toxicities and effects of the ribozyme in an *in vivo* setting by using the SCID-Hu model in collaboration with D. Mosier (35). Human PBLs are transduced *in vitro* as in our prior studies and subsequently tested for resistance to viral challenge *in vivo* in the mice. The status of these experiments will be summarized at the time of the RAC meeting.

Finally, we note that our ribozyme project collaborator, Dr. Jay Rapapport (NIH) has found no evident toxicity of this ribozyme in transgenic mice (Rapapport J, personal communication).

D. Previous Human Gene Therapy Trials. We have designed this human trial with the benefit of a great wealth of data acquired in the past three to five years by other groups of investigators that support not only the general safety of retroviral vector-based gene therapy in humans but also the particular vector and packaging system we will use (2,10, 21-25,37,42, 46,47, 55). Most human gene transfer trials approved by the RAC and all of its subcommittees to date have employed retroviral vectors for *ex vivo* gene transfer (2, 55).

LNL6, the vector we are employing to deliver the ribozyme gene, was used in the first human gene transfer trial (37) to transfer the NeoR marker gene to tumor-infiltrating lymphocytes. The first actual human gene therapy trial (i.e. not solely a gene-marking trial) began in September, 1990 and involved retroviral vector-mediated transfer (by LASN, a modified LNL6-based vector) of the adenosine deaminase (ADA) gene into the lymphocytes of a patient with ADA deficiency (2). Toxicity referable to the vector or the packaging line was searched for and was not observed in these or subsequent trials (2, 55).

Furthermore, all approved studies have used the packaging line we will use, PA317 (2,23). In the human trials to date, none of the production batches of retroviral vectors and none of the human patients have tested positive for replication-competent helper virus (2). We are aware that thymic lymphoma has developed in three of eight rhesus monkeys given bone marrow infected with a retroviral stock known to be heavily contaminated with replication-competent virus (2, 38,39). Although the disease observed in these monkeys was not observed in previous studies of infection of

cynomologous macaque and rhesus monkeys with amphotropic murine helper virus (41), a broad consensus exists that stringent testing for helper virus is necessary for human trials (2,42,55) and we plan the comprehensive studies now standard in gene therapy protocols (explained in part B of POTENTIAL RISKS & RISK MANAGEMENT PROCEDURES below).

Two other trials approved for treatment HIV disease have guided our preparation of this proposal. One is a marker study involving retroviral vector-mediated transfer of a marker gene to HIV antigen-specific T cells (2). The other study is a protocol involving retroviral vector transfer of a trans-dominant rev protein to peripheral blood lymphocytes of HIV-infected subjects (G. Nabel, personal communication).

We therefore believe that these considerations and the potent anti-viral effects we have observed in primary T cells provide ample justification for phase I testing of the safety and effects of this ribozyme in human subjects with HIV infection.

2 . FACILITIES

The *in vitro* manipulations of patient lymphocytes, including transduction, selection and expansion of cells will be performed in the UCSD Clinical Sciences Building, a new building with a full range of needed equipment, including two large Biosafety Level 3 (BL-3) containment facilities, tissue culture facilities and computer analytic capabilities. One floor of the building (approxamately 5000 square feet) is reserved for sole use by the Wong-Staal laboratory and one of the investigators (EMP) is supervisor of the BL-3. Manipulations of HIV-infected cells after harvesting and prior to re-infusion will be carried out with adherence to standard BL-3 containment practices with which the investigators have many years of cumulative experience.

Evaluation of patients, harvesting of patient lymphocytes and re-infusion of gene-transduced lymphocytes will be carried out in the UCSD Clincal Research Facility, the UCSD Medical Center and the Veterans Administration Medical Center, San Diego, CA.

3. ESTIMATED DURATION OF STUDY

One year of close clinical follow-up and laboratory analyses is planned for the primary experimental observations . We plan to continue long-term follow-up of patients with

subsequent laboratory evaluations as indicated by the results of the trial. The informed consent form will ask for permission for autopsy.

4. SUBJECT POPULATION

Four to six carefully selected patients with Human Immunodeficiency Virus-1 (HIV-1) infection will be enrolled. The following eligibility criteria will be used:

4.1 Inclusion Criteria

a. HIV-1 infection documented by ELISA and confirmatory western blot.
b. Age $\geq$ 18 and $\leq$ 65 years.
c. Patients of either sex are eligible. All patients with reproductive potential, male or female, must agree to use an effective method of contraception during the study period.
d. The patient must be able to provide informed consent.
e. Karnofsky score > 80% at enrollment.
f. The patients must take zidovudine (AZT). This criterion is included after extensive debate among the authors of this proposal and with acknowldegement of its inclusion in one previously RAC-approved protocol; for a discussion of our reservations about mandating AZT use, please see Appendix 1. Briefly, the rationale for the criterion is that AZT would inhibit replication-competent murine helper virus, a risk also addressed comprehensively by other measures (see sections on risk management). It is understood that AZT intolerance (resulting from toxicities including, but not limited to, bone marrow supression, intractable nausea, myopathy) could occur on study in any given patient and necessitate withdrawal of the drug.

4.2 Exclusion Criteria:

a) Laboratory parameters:
   i. $CD4^+$ cell count < 250 cells/mm^3. This baseline CD4 level will be established by two or more measurements at least two weeks apart.
   ii. Hematocrit < 30%.
   iii. Platelet count < 50,000/mm^3.
   iv. White blood cell count < 3000/mm^3.

v. Absolute neutrophil count < 1000/mm^3.

vi. Creatinine > 2.0 mg/dl.

vii. Total bilirubin > 2.0 mg/dl.

viii. SGOT and SGPT > 4 times upper limit of normal reference value.

b) Current or previous AIDS (Acquired Immunodeficiency Syndrome)-defining opportunistic infection (revised 1993 CDC criteria, ref. 1), Kaposi's Sarcoma, lymphoma, cervical or other carcinoma, or hairy leukoplakia.

c) Pregnant or nursing women.

d) An acute infectious illness within one month of initiation of treatment.

e) Any other serious chronic illness including, but not limited to: diabetes, chronic active hepatitis, sarcoidosis, active autoimmune disease (such as rheumatoid arthritis, systemic lupus erythematosus, Reiter's Syndrome, inflammatory bowel disease, thyroiditis, etc.), coronary artery disease, cardiomyopathy, chronic obstructive pulmonary disease, dementia.

f) Patients receiving systemic glucocorticosteroids.

g) Allergy to penicillin, streptomycin or amphotericin B.

h) Active alcohol abuse or drug abuse or psychiatric impairment or occupational or personal circumstances that in the opinion of the investigators would prevent compliance with the protocol.

i) Patients who have undergone alternative non-FDA approved treatment within four weeks of initiation of the protocol.

5. GENE TRANSFER PROTOCOL

A. **Enrollment:** Subjects who meet the eligibility requirements will have the study explained in detail to them by Dr. Eric Poeschla or Dr. David Looney (designated at points in the protocol as investigating physicians). Subjects will then be asked to give witnessed, informed consent in writing (see Sections 10 and 11 below).

B. Pre-treatment evaluations:

1. Complete medical history, complete physical examination and performance status assessment by a study physician.
2. CBC, differential count, platelet count, PT, PTT.
3. Chemistries: glucose, calcium, total protein, albumin, uric acid, phosphate,
4. BUN, creatinine.
5. Urinalysis.
6. Pregnancy test (where indicated).
7. Electrocardiogram, chest x-ray.
8. p24 antigen level.
9. CD4 counts.
10. Quantitative PCR for viral load RNA analysis.
11. Cryopreservation of 10 ml of serum and PBMC derived from 45 ml of peripheral blood.

C. Gene Transfer Methods:

In the gene transfer procedures outlined below, three measures will be employed to avoid activation of latent HIV present in patient peripheral blood lymphocytes. The non-nucleoside reverse transcriptase (RT) inhibitor nevirapine and CD4-pseudomonas exotoxin (CD4-PE40), will be included during the *ex vivo* expansion and transduction of patient lymphocytes. The latter agent is a fusion protein consisting of the HIV-envelope binding region of human CD4 linked to the translocation and ADP-ribosylation domains of *Pseudomonas aeruginosa* exotoxin A (50). Binding of one toxin domain allows entry of the lethal second domain into the cell; CD4-PE40 has been shown to selectively eliminate infectious HIV from cultures of human T cells lines when used in concert with reverse transcriptase inhibitors (51). Nevirapine belongs to the non-nucleoside RT inhibitors, a group of structurally diverse compounds that non-competitively inhibit HIV RT and viral replication at nanomolar concentrations, typically have therapeutic indices (assayed in cultured cells) of 1000 to 1, and are highly specific for HIV-1, lacking activity against HIV-2, SIV or murine reverse transcriptases (27,52,53). This synergistic combination of an agent selectively virustatic for HIV-1 and an agent selectively cidal for gp120-expressing cells will prevent amplification of HIV during the protocol without interfering with transduction by the PA317-packaged vector.

Finally, we will assay for gag expression in the expanded cells by a commonly used and highly sensitive HIV-1 p24 antigen capture assay (Coulter). Other novel reverse-transcriptase inhibitors and antivirals (27, 54) have potential application to this problem in future protocols.

1. Harvesting of patient PBMC for *ex vivo* stimulation

Following completion of baseline data acquisition, study subjects will undergo leukopheresis or phlebotomy of not more than 8 ml/kg per 4 week interval. Fresh peripheral blood mononuclear cells will be separated from erythrocytes and neutrophils by Ficoll-Hypaque density gradient centrifugation. The PBMCs will then be washed, and depleted of CD8+ cells utilizing murine anti-human CD8-monoclonal antibody coated flasks (Applied Immune Sciences CELLectorTM Flasks). Each flask will be loaded with 30 mls of 2-3 x 10^6 cells/ml and incubated at room temperature for one hour. Non-adherent cells are to be stimulated in CM with 10 ng/ml OKT3 antibody [CM = AIM-V serum-free media (Gibco) with 2mM glutamine, 50 U/ml penicillin, 50 ug/ml streptomycin, 2.5 ug/ml Fungizone, IL-2 1000 U/ml and the HIV-1 reverse transcriptase inhibitor nevirapine (Boerhinger-Mannheim) at 40 nM and CD4-pseudomonas exotoxin (Upjohn)]. Cellular phenotype is assessed by flow cytometry prior to expansion.

2. Expansion in IL-2. OKT3-activated cells are resuspended in fresh CM containing 60 U/ml of IL-2 (Cetus, Emeryville, CA) at 1-2 x 10^5 cells/ml and expanded in 3000 ml culture bags (Lifecell PL732, Fenwal, Deerfield, IL) each containing about 500-1000 ml media. Cells are grown to maximum density (about 1-2 x 10^6 cells/ml). Expansion is estimated to take 3-4 days.

3. Transduction. Two identical aliquots of cells will be transduced simultaneously, one with the ribozyme-bearing vector and with the control vector (vector lacking the ribozyme). Frozen viral supernatants will be stored at -80°C. On the day of transduction, aliquots will be thawed and passed through 0.45 micron filters. Cells will be resuspended at a concentration of 10^5 per ml in a transduction mixture having 50% CM and 50% viral supernatant (MOI of 1 to 3) supplemented with protamine sulfate (49) at a final concentration of 5 μg/ml. After 4-8 hours incubation at 37 degrees C, cells will be washed 3 times in CM and introduced into tissue culture bags for large scale expansion for therapy.

4. Growth and selection of transduced lymphocytes Transduced lymphocytes will be expanded and selected by culture in CM with 400 ug/ml G418 for 7-8 days. Efficiency of selection will be monitored in parallel by viability of non-transduced cultures. After selection is complete, G418 will be removed and the cultures expanded up to 3 X 10^9/kg for reinfusion.

5. Infusion of transduced, expanded autologous lymphocytes. The transduced cells will be harvested, washed, and resuspended in normal saline. The final cell preparation will be filtered through a platelet filter and transferred into a syringe or transfusion pack for infusion. Patients will be admitted to the Clinical Research Facility or the inpatient service of the V.A. Medical Center. Interim history and physical examination will be performed by a study physician. Intravenous catheterization with standard sterile technique will be performed and good venous return and absence of infiltration will be verified immediately before infusion. The infusion will be of not more than $5x10^8$ cells/kg of body weight. (The dose target per infusion in the ADA Clinical protocol, ref. 22, was 1-3 x 10^9 per kg). Total volume of infused cells should not exceed 10 ml/kg of body weight. After an initial test infusion of 1-5% of the total volume, cells will be infused over the next 60-120 minutes. During infusion, the cell suspension will be mixed gently approximately every 5 minutes while the patient is being observed for acute and subacute toxicity. Vital signs will be monitored before infusion and every 15 minutes during and 2 hours after infusion or until the patient is stable. The infusion will be terminated if systolic blood pressure falls below 80 mm Hg, oxygen saturation falls below 90%, or other evidence of major systemic toxicity occurs. Patients will have nursing observation for 24 hours after infusion; patients will be discharged after 24 hours if there are no complications. A blood sample or samples will be drawn prior to discharge for initial studies detailed in the following section.

D. Follow-up studies

The patients in this study will have direct access to an investigating physician via telephone or pager. The following patient visits and data collection are planned (on the schedule indicated in table 1 below):

1. Interim history and physical examination by an investigating physician.

2. Isolation of PBLs for:

a. Limiting dilution quantitation and DNA PCR to assess transduced cell survival *in vivo*. PCR primers will be chosen to acheive amplification specific for the ribozyme-containing vector and control vector. The longevity of the transduced cells, the primary experimental variable to be monitored in this trial, is difficult to prospectively estimate. Nevertheless, we note that in the ADA trial a large number of gene-corrected T cells survived in the first patient for over six months after cell infusions were stopped, a result that confounded pre-trial concerns that cells would have a severely short lifespan (2).

b. RNA PCR to assess ribozyme expression in vivo.

c. CTL assay.

d. Virus isolation.

e. Phenotype of transduced cells in vivo.

3. CD4 count

4. p24 antigen level.

5. Complete blood counts and standard chemistries (SMA-20).

TABLE 1

Week:	1	2	3	4	8	12	16	20	24	32	52
Test											
*1.	X	X	X	X	X	X	X	X	X	X	X
2-a.	X	X		X		X		X		X	X
2-b.	X			X		X		X		X	X
2-c.	X					X					X
2-d.				X							X
2-e.	X					X					X
3.	X			X		X					X
4.	X			X		X					X
5.	X	X	X	X	X	X	X	X	X	X	X

*Numbers refer to designations in immediately preceding section

6. POTENTIAL RISKS & RISK MANAGEMENT PROCEDURES

6.1 Toxicity Criteria & Reporting of Adverse Effects

A. Monitoring of adverse effects. Subjects will be clinically evaluated at frequent intervals as detailed above (PROJECT PROTOCOL). All side effects will be graded using standard criteria (see attached). Subjects with recurrent grade 3 or greater toxicity will be removed from the protocol and replaced by another subject. If recurrent grade three toxicity is observed in more than one subject, the trial will be suspended.

B. Reporting of Adverse Effects. Adverse reactions will be reported to the UCSD Human Subjects Committee and in writing within 10 working days to Investigational Drug Branch, P.O. Box 30012, Bethesda, MD 20824, Phone 301-496-7957, and include all fatal events, all life-threatening events (Grade 4) which may be due to drug administration, or the first occurrence of any previously unknown clinical event (regardless of grade).

6.2 Potential Risks of Gene Therapy with Ribozymes

A. Insertional mutagenesis. Retroviruses insert randomly in the genome and the theoretical risk exists that transduction of lymphocytes with a retroviral vector could give rise to a clone or clones of malignant cells. This risk is considered low, however (2, 10,24,42,55).

B. Toxicity specific to the ribozyme. There is an even smaller, wholly theoretical risk that the ribozyme could cleave or have anti-sense activity against the mRNA of an unknown gene having tumor-suppressor activity and thus cause malignancy. Even accounting for wobble at certain bases, the probable frequency of a sequence homologous to the leader-sequence ribozyme can be calculated to be about one per eukaryotic genome. Since only a small percentage of the genome is expressed and an even smaller portion of such messages can be expected to have tumor-suppressor activity, the likelihood of carcingenesis resulting from cleavage of a human mRNA is vanishingly small. Carcinogenesis has not been apparent in previous human gene therapy trials (2,42,55); this risk, which is discussed in more detail above in OBJECTIVES AND BACKGROUND, is

very small for any one patient, and is acceptable in the context of treatment for AIDS, an invariably lethal illness.

C. Replication-competent helper viruses. Minimization of the risk (discussed in detail above in OBJECTIVES AND BACKGROUND) of pathological replication-competent retroviruses arising through recombination events or contamination is designed into all aspects of development. As discussed above, packaging cell lines express structural and other genes needed for production of packaged vectors from separate constructs. The signal for packaging is contained only in the vector, not in the packaging cell line. Moreover, in previous human gene therapy trials employing this vector, no difficulties with helper virus have emerged (2,24,25,42,55). FDA recommended procedures for screening all retroviral supernatant lots for adventitious virus will be followed. Tests that will be done on supernatants used for patient PBMC transduction include: titre, sterility (see cultures below), marker rescue assay, S^+L^- assay for ecotropic virus, S^+L^- assay for xenotropic virus, S^+L^- assay for amphotropic virus, 3T3 amplification by standard methods, titre assay for HIV. PCR and marker rescue assays will also be done on patient PBLs after treatment to monitor for the unlikely event of replication-competent virus.

While *in vivo* rescue of vector by and/or recombination with HIV are perhaps remotely theoretically possible, there is no experimental support or precedent for such an event which is extraordinarily unlikely to happen or to result in a pathogenic entity.

D. Mutation of Germ Line. Without replication competent helper virus (screened as in part B above), no risk of germ line transduction exists for this kind of *ex vivo* gene transfer.

E. Aminoglycoside Antibiotic Resistance. The NeoR gene product, neomycin phosphotransferase, confers cellular resistance to neomycin by phosphorylating the 3' hydroxyl group of the aminohexose I of neomycin and its analogs. Although amikacin can be inactivated by this enzyme, the more commonly used gentamicin and tobramycin do not contain an hydroxyl at the 3' position and are not inactivated. In any case, transduction of the NeoR gene will occur into only a fraction of peripheral blood cells. Therefore, no adverse consequences are foreseeable for the use of aminoglycoside or any other antibiotics in patient care.

F. Autoimmune Disease. A wholly theoretical risk exists that expression of the protein neomycin phosphotransferase could lead to an immune response that would be generalized to other (non-transduced cells) in the body. The extensive existing literature includes no evidence for such a phenomenon occurring in animals, nor has such an effect been observed in humans in gene therapy trials to date (2,42,55).

G. Risks related to harvesting of cells and re-infusion. The risks of venipuncture include bruising and minor bleeding. The amount of blood that will be phlebotomized should not cause volume depletion hemodynamically significant enough to result in syncope or to significantly exacerbate symptoms of mild chronic anemia attendant to HIV infection (initial eligibility hematocrit cut-off is 30%). Nevertheless, patients will be observed for at least one hour following phlebotomy and examined for clinically significant orthostatic blood pressure changes.

Since harvested patient cells will undergo expansion and manipulation in vitro, there is a risk of microbial contamination with bacteria, mycoplasma, fungi or viruses. All procedures will be optimized to maintain sterility of patient cells. Monitoring of cells prior to re-infusion will include: viability (at least 70% trypan blue exclusion), culture for helper virus as indicated above, surveillance for aerobic and anaerobic bacterial and fungal contamination by culture in thioglycolate broth and on blood agar and chocolate agar. A probe hybridization test will be used to test for mycoplasma and other prokaryotic organisms. The standard limulus assay is planned to test for endotoxin. A gram's stain of the final cell pellet will be done prior to infusion to confirm the absence of organisms. Finally, to insure that all components of the protocol are in place, we plan to dry-run the procedure at least once on human PBLs before the first patients are treated.

H. Confidentiality. Every attempt will be made to preserve strict confidentiality and privacy throughout the study. No patient identifiers will be included in case report forms or other communications or in laboratory labelling. Patients will be assigned confidential study identifiers which will be used in reports and publications as well as in laboratory processing of patient material. Standard procedures for any communication with the press will be followed including no identification of the patient or details of their medical problems other than those obvious from the study entry criteria.

7. POTENTIAL BENEFITS

This phase 1 study is not designed to achieve immune reconstitution or definitive therapy of HIV disease. However, infusion of some $CD4^+$ lymphocytes resistant to productive HIV infection could have a beneficial effect on the course of HIV disease. Since stem cell transduction is not the goal of the current project, the possibility is negligible that circulating stem cells could be transduced during this protocol and give rise to a persistent *in vivo* clone or clones of HIV-1 resistant lymphocytes. However, the potential benefit to future patients of optimizing ribozyme gene therapy for HIV infection and other diseases is enormous.

8. RISK-BENEFIT RATIO

Although a few long-term (more than twelve years) healthy survivors of established HIV infection have been reported, this condition is virtually always relentlessly progressive and fatal. The risks involved are discussed above and are small but finite. The potential knowledge gained (which in the absence of a good animal model for the disease can be gained in no other way) is tremendous. Therefore the risk-benefit ratio appears quite favorable.

9. EXPENSE TO SUBJECTS

No additional expense will be incurred by subjects participating in this study. This study will not provide routine medical care.

10. INFORMED CONSENT

The investigating physicians will carefully explain the study's goals, risks, potential discomforts and limitations to each prospective subject. After the patient has had at least one week to read the consent form, ask questions of the study physicians and consult his or her family, other physicians or sources of advice as he or she wishes, a signed, witnessed consent form will be obtained by an investigating physician. Recruitment of subjects will be through referrals based on individual physician-patient encounters in the UCSD system. No written advertising for patients is contemplated. The consent document includes a request for autopsy at the time of the patient's death.

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44. Morgan RA; Cornetta K; Anderson WF. Human Gene Therapy, 1990 Summer, 1(2):135-49.
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46. Blaese RM; Culver KW. Immunodeficiency Reviews, 1992, 3(4):329-49.
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54. Craig JC; Duncan IB; Hockley D; Grief C; Roberts NA; Mills JS. Antiviral Research, 1991 Dec, 16(4):295-305.
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University of California - San Diego

Consent to Act as a Research Subject

Drs. Flossie Wong-Staal and her colleagues are conducting a study to find out more about gene therapy for the treatment of HIV and AIDS (Acquired Immunodeficiency Syndrome). The study is entitled "Ribozyme Gene Therapy for HIV." You have been asked to participate because you have infection with the Human Immunodeficiency Virus (HIV).

The purpose of this study is to test the safety and effects of an experimental treatment for HIV. The treatment is called "ribozyme gene therapy".

This therapy is called "gene therapy" because the object of it is to give your cells a new gene, the gene for the ribozyme. Ribozymes are pieces (molecules) of genetic material ("genes") that have been popularly called "molecular scissors". Like a scissors, their function is to cut. Ribozymes cut other pieces of genetic material. The ribozyme that will be used to treat your cells cuts the genetic material of HIV. In this way, we hope the treated cells will become protected against HIV when they are back in your body because the ribozyme can cut the virus's genetic material inside the cells.

Tests we have done with cells in the laboratory has shown that the ribozyme does help to protect cells growing in laboratory dishes from HIV. In this study, we will study what happens to you and to your cells when they are put back into your body after being given the ribozyme. That way we can find out whether what works in cells in laboratory dishes will work in cells when they are in your body. We will find out if this can be done safely and whether or not there are any side effects.

If you agree to participate in this study, the following will happen to you:

1. Dr. David Looney or Dr. Eric Poeschla will take your complete medical history and perform a complete physical examination.

2. You will have blood tests and x-rays to determine if you will be able to complete the study.

3. If you are eligible for the study, you will come into the clinic and have about 12-18 ounces (a typical can of soda is 12 ounces) of blood drawn from a vein in your arm. The exact amount will depend on your weight (roughly 6 ounces for each 50 lbs. of your weight). We will watch you for an hour or so to make sure you tolerate the blood drawing without any problems and, if so, you will then be able to go home on the same day.

4. In the laboratory some of your white blood cells (your T cells) will be separated from the rest of your blood and placed in laboratory dishes. There they will be treated in various ways to make them grow and to keep any HIV in them from spreading in the dishes. After the cells have multiplied in number, they will be treated with the ribozyme.

5. While your cells are being given the new ribozyme gene in the laboratory and allowed to multiply (a few days to weeks), you will be kept informed regularly about the schedule of what needs to be done next. You will be notified when the cells are ready to be put back into you. You will be admitted to the hospital or the UCSD Treatment Center and a needle (an intravenous line or "I.V.") will be inserted in your vein. Your genetically modified cells will then be infused back into you through the needle over a period of a few hours.

6. Follow-up studies. Because this is a new and experimental treatment, we will be observing you closely during and after the treatment to determine if there are side effects to the therapy. We will also closely monitor the effects of this treatment on your immune system. We will do this is through a combination of regularly talking to you about any symptoms or problems you may have, examining you in the clinic and by doing a variety of blood tests. It will take about a year of such visits to complete your participation in the study, at first every week or so for a month and then about every month or so. Small amounts of blood for testing will be drawn at each visit.

Possible Risks, Discomforts and Side Effects

Participation in this study may involve some added risks or discomforts.

These include:

1. You may experience discomfort or bruising from blood drawing and intravenous infusions. Sometimes people get lightheaded or even faint after blood drawing. There is also the remote possibility of infection at the blood drawing site or I.V. site.

2. Although the DNA that will be inserted in your cells is considered safe, there is a remote chance that inserting the gene into the DNA of your cells could cause cancer to develop, which would create additional problems and may require additional treatments. Laboratory studies and previous studies of similar gene therapy in people suggest that this possibility is very low. However, this is a new procedure and we do not know whether cells could become abnormal after a long time.

3. The inserted DNA has a gene that can cause a few antibiotics to be inactivated in the test tube. This gene will not likely be active in any appreciable amount in your body and many other antibiotics that are not inactivated by it will be available for treating any potential bacterial infections. Therefore it is unlikely to significantly interfere with giving you effective antibiotics for any infections you may develop in the future.

4. Although many efforts will be made to ensure no bacteria, viruses or other kinds of infections get into your cells while they are being genetically modified outside your body, a small possibility exists that such contamination could occur and cause harmful or even fatal infection when the cells are put back.

5. Although previous animal and human studies suggest it is unlikely, there is a possibility that having a new gene in some cells of your body could provoke a response by your immune system against those and other cells in your body.

If you are injured as a result of participation in this research, the University of California will provide any medical care you need to treat those injuries--except when they are a consequence of research procedures designed to benefit you directly. The

University will not provide any other form of compensation to you if you are injured. You may call 619-534-4520 for more information about this or to report research-related problems.

Your participation in this study is entirely voluntary. You may refuse to participate or withdraw from the study at any time and for any reason without jeopardizing your future health care in any way at this institution.

Because only a relatively small number of cells compared to the total number of cells in your body will be transferred back to you in this study, it is not known if you yourself will benefit. However, the knowledge gained from this study may benefit others in the future.

There will be no added costs to patients participating in this study. This study will not pay for routine medical care.

Alternative Therapies

Neither HIV infection nor AIDS can be cured with presently known treatments. Alternative treatments available to you that may have some beneficial effects are medicines such as zidovudine (AZT), didanosine (ddI) and zalcitabine (ddC). Medicines (such as antibiotics), surgery or radiation therapy can be used to treat some complications of HIV infection. All of the above treatments, however, can and will be used if they are indicated for you according to standard clinical practice during this study--they will not interfere with ribozyme gene therapy. In other words, ribozyme gene therapy will be used in addition to these other established methods of treatment. You should also know that other experimental treatments for HIV are being investigated elsewhere and you can be referred to physicians who are conducting such trials. At some later time, should new alternatives for treatment of HIV become available, they will be discussed with you and offered to you. You also have the option to receive no treatment at this time.

Confidentiality

Every attempt will be made to preserve strict confidentiality and privacy throughout the study. When results of a study like this are reported in medical journals or at meetings the patients taking part are never identified. Research records will be kept confidential to the extent provided by law and are made available for review by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study records.

If you are female and capable of child-bearing, a sample of urine will be collected before the study is begun in order to be sure as possible that you are not pregnant. Both men and women who participate must agree to use an effective contraception method (such as condoms, the pill, diaphragm, or intrauterine device) to prevent reproduction for the duration of the study since it is possible that the treatment being tested could cause harm to an unborn child. Whether or not they also use a method such as the pill or diaphragm to ensure prevention of pregnancy, all people with HIV infection should always use a condom during sex to prevent transmission of HIV.

If you agree to participate in this study you are asked also to consent to have an autopsy performed on your body after your death. The reason for this request is that much information that is of potential benefit to future recipients of gene therapy can be learned that way.

SIGNATURE

Dr. _____ has explained the purpose and risks of this study to me and answered all of my questions.

If I am female, I am and will not become pregnant during this study.

I have asked for and received all information I need to use effective methods of contraception and will use such methods for the duration of the study.

I hereby grant permission for a full autopsy following my death. I also stipulate that this permission may not be rescinded by any relatives, next of kin, legal guardian or representative or holder of power of attorney.

My blood will be used in research to be conducted by University of California personnel. The cells and fluids from my blood, or their derivatives, or material obtained at autopsy may have significant therapeutic and commercial value. I consent to such uses. I hereby release the University of California from any liability for such use.

If I have other questions or research related problems I may call Dr. _____ at _____

I have received a copy of this consent document to keep and a copy of "The Experimental Subject's Bill of Rights."

I have read, understand and agree with all four pages of this consent document.

I hereby agree to participate.

_____	_____	_____
Subject's Signature	Witness's Signature	Date

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Flossie Wong-Staal, Ph.D.	Professor of Medicine and Biology

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of California, Los Angeles, CA	B.A.	1968	Bacteriology - (Magna Cum Laude)
University of California, Los Angeles, CA	Ph.D.	1972	Molecular Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

1969-1970 Teaching Assistant, University of California, Los Angeles, California
1970-1972 Research Assistant, University of California, Los Angeles, California
1972-1973 Post-doctoral Fellow, University of California, San Diego, California
1973-1975 Fogarty Fellow, National Cancer Institute, Bethesda, Maryland
1975-1976 Visiting Associate, National Cancer Institute, Bethesda, Maryland
1976-1978 Cancer Expert, National Cancer Institute, Bethesda, Maryland
1978-1981 Senior Investigator, National Cancer Institute, Bethesda, Maryland
1982-1989 Chief, Molecular Genetics of Hematopoietic Cells Section, National Cancer Institute, Bethesda, Maryland
1990-Present Florence Seeley Riford Chair in AIDS Research; Professor of Medicine and Biology, University of California, San Diego, La Jolla, California

Societies

American Society for Virology, Charter Member

Honors and Awards

1967-1968 Regents Scholarship, UCLA
1968 Phi Beta Kappa
1968 Regents Fellowship, UCLA
1972 Women Graduates of the Year Award, UCLA
1985 Visiting Professorship, Institute of General Pathology; First University of Rome, Italy
1987 Outstanding Scientific Award, The Chinese Medical and Health Association
1991 The Excellence 2000 Award, United States Pan Asian American Chamber of Commerce and the Organization of Chinese American Women

Editorial Boards

Gene Analysis Techniques, 1984 - Present
Cancer Letters, 1984 - Present
Leukemia, 1987 - Present
Cancer Research - 1987
Journal of AIDS Research and Human Retroviruses (Section Editor), 1987 - Present
DNA and Cell Biology (Section Editor) 1987 - present
Microbial Pathogenesis, 1987 - 1990

CONTINUATION PAGE: STAY WITHIN MARGINS INDICATED

AIDS: An International Journal, 1987 - Present
International Journal of Acquired Immunodeficiency Syndrome, 1988 - Present
Oncogene, 1988 - Present
Journal of Virology - 1990 - Present

PUBLICATIONS

1. Hahn, B.H., Shaw, G.M., Arya, S.K., Popovic, M., Gallo, R.C., Wong-Staal, F.: Molecular cloning and characterization of the HTLV-III virus associated with AIDS. Nature 312: 166-169, 1984.
2. Shaw, G.M., Hahn, B.H., Arya, S.K., Groopman, J.E., Gallo, R.C., and Wong-Staal, F.: Molecular characterization of human T-cell leukemia (lymphotropic) virus type III in the acquired immune deficiency syndrome. Science 226: 1165-1171, 1984.
3. Ratner, L., Haseltine, W., Patarca, R., Livak, K.J., Starcich, B., Josephs, S.F., Doran, E.R., Rafalski, J.A., Whitehorn, E.A., Baumeister, K., Ivanoff, L., Petteway, S.R., Jr., Pearson, M.L., Lautenberger, J.A., Papas, T.S., Ghrayeb, J., Chang, N.T., Gallo, R.C., and Wong-Staal, F.: Complete nucleotide sequence of the AIDS virus HTLV-III. Nature 313: 277-284, 1985.
4. Fisher, A.G., Collalti, E., Ratner, L., Gallo, R.C., and Wong-Staal, F.: A molecular clone of HTLV-III with biological activity. Nature 316: 262-265, 1985.
5. Arya, S.K., Guo, C., Josephs, S.F., and Wong-Staal, F.: Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science 229: 69-73, 1985.
6. Wong-Staal, F., Shaw, G.M., Hahn, B.H., Salahuddin, S.Z., Popovic, M., Markham, P., Redfield, R., and Gallo, R.C.: Genomic diversity of human T-lymphotropic virus type III (HTLV-III). Science 229: 759-762, 1985.
7. Wong-Staal, F., Shaw, G.M., Hahn, B.H., Salahuddin, S.Z., Popovic, M., Markham, P., Redfield, R., and Gallo, R.C.: Genomic diversity of human T-lymphotropic virus type III (HTLV-III). Science 229: 759-762, 1985.
8. Feinberg, M.B., Jarrett, R.F., Aldovini, A., Gallo, R.C., and Wong-Staal, F.: HTLV-III expression and production involve complex regulation at the levels of splicing and translation of viral RNA. Cell 46: 807-817, 1986.
9. Fisher, A.G., Ensoli, B., Looney, D., Rose, A., Gallo, R.C., Saag, M.S., Shaw, G.M., Hahn, B.H., and Wong-Staal, F.: Biologically diverse molecular variants within a single HIV-1 isolate. Nature 334: 444-447, 1988.
10. Vaishnav, Y.N., Vaishnav, M., and Wong-Staal, F.: Identification of a nuclear factor that binds simultaneously and proximally with the HIV Rev protein to the Rev Response Element (RRE). New Biol. 3: 142-150, 1991.
11. Daefler, S., Klotman, M., and Wong-Staal, F.: Specific interaction of the HIV-1 Rev protein with its target RNA sequence. Proc. Natl. Acad. Sci. USA 87: 4571-4575, 1990.
12. Vaishnav, Y. and Wong-Staal, F.: The Biochemistry of AIDS. Ann. Rev. of Biochem. 60: 577-630, 1991.
13. Steffy, K., Kraus, G., Looney, D.J., and Wong-Staal, F.: Role of the fusogenic peptide sequence in syncytium induction and infectivity of human immunodeficiency virus type 2. J. of Virol. , in press.
14. Ojwang, J., Hampel, A., Looney, D., Wong-Staal, F. and Rappaport, J.: Inhibition of Human Immunodeficiency Virus Type-1 (HIV-1) expression by a Hairpin Robozyme. Proc. Natl. Acad. Sci. USA, 89: 10802-10806, 1992.
15. Yu, M., Ojwang, J., Yamada, O., Hampel, A., Rappaport, J., Looney, D., and Wong-Staal, F.: A hairpin ribozyme inhibits expression of diverse strains of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci., USA, 90: 6340-6344.

GRANT NUMBER: _____

BIOGRAPHICAL SKETCH

Give the following information for all new key personnel, consultants, and collaborators.
Copy this page for each person.

NAME	POSITION TITLE
Eric Murnane Poeschla	Post-doctoral Fellow

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Franklin and Marshall, Lancaster, Penn.	B.A.	1980	
Yale University, New Haven, Conn.	M.D.	1985	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individual who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Post-doctoral Training

1990-93 Post-doctoral Fellow in Medicine (Infectious Diseases), University of California, San Diego (7/90-present)
1988-89 Staff Physician, Simbu Province Hospital, Kundiawa, Papua, New Guinea.
1986-88 Resident in Internal Medicine, University of California, San Francicso.
1985-86 Intern in Internal Medicine, University of California, San Francisco.

Licensure and Board Certification

1988 Diplomate, American Board of Internal Medicine.
1986 Licensed as Physician and Surgeon in California.
1985 Diplomate, National Board of Medical Examiners.

Honors and Awards

1991 UCSD Physician-Scientist Award
1985 Peter F. Curran Prize for Yale M.D. Thesis-judged best in the class.
1980 Phi Beta Kappa
1980 Frederick C. Schiffman Prize for excellence in Humanities and Social Sciences.
1980 Bradford S. Kline Memorial Award in Biology

Publications

1. Kelly G, Poeschla EM, Barron HV, Forrest JN.: A_1 a denosine receptors inhibit chloride transport in the shark salt gland. Dissociation of inhibition and cycle AMP. J of Clin Invest 85 (5):1629-36, 1990.

2. Ukena D, Poeschla EM.: Effects of N-ethylmaleimide on adenosine receptors of rat adipocytes and human platelets. N-S Arch Pharmacol 327: 247-253, 1984.

3. Ukena D, Poeschla EM, Hutteman E, Schwabe U.: Guanine nucleotide and cation regulation of radioligand binding to R_i adenosine receptors of rat adipocytes and human plateles. N-S arch Pharmacol 326: 241-47, 1984.

4. Forrest JN, Poeschla EM, Rieck D.: Ribose specific (A_2) adenosine receptors mediate cyclic AMP accumulation and chloride transport in the elasmobranch salt gland.: Regulatory Function of Adenosine. Berne RM, Rall TW, Rubio R., Martnius Nijhoff, Pub., Boston, p. 511-4, 1983.

5. Poeschla EM, Kelly G, Boyer P, Forrest JN.: Evidence for an inhibitory adenosine receptor in the salt gland of Squalus acanthias. Bull Mt. Desert Island Bio Lab 22: 519-22, 1982.

6. Poeschla EM, Kelly G, Link D, Forrest JN.: Theophylline inhibits cyclic AMP accumulation and chloride secretion due to stimulatory adenosine receptors in the shark salt gland. Bull Mr. Desert Island Bio Lab 22: 515-18, 1982.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
David J. Looney, M.D.	Assistant Professor of Medicine, In Residence

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Washington University, St. Louis Mo.	B.S.	1974	Physics
University of Missouri, School of Medicine, Columbia, MO.	M.D.	1979	Medicine
Letterman Army Medical Center, San Francisco, California	Internship& Residency	1979-1982	Internal Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Postdoctoral Training:

Brooke Army Medical Center, San Antonio, Texas	Subspecialty Fellowship	1984-1986	Infectious Diseases
San Antonio State Chest Hospital and University of Texas Health Science Center	Guest Researcher	1984-1986	Cellular Immunology
Walter Reed Army Institute of Research, Washington, D.C.	Subspecialty Training	1986	Tropical Medicine
Laboratory of Tumor Cell Biology, NCI, NIH, Bethesda, MD	Guest Researcher	1986-1989	Molecular Virology, Laboratory of R.C. Gallo & F. Wong-Staal

Professional Experience:

Jul 1989-Nov 1992 : University of California San Diego and Veterans Administration Medical Center. Assistant Professor of Medicine In Residence and Infectious Diseases and Internal Medicine Staff Physician.

Sep 1989 - Jun 1989 : Uniformed Services University of Health Sciences School of Medicine. Assistant Professor of Medicine.

Jul 1986 - Jun 1989 : Walter Reed Army Medical Center. Infectious Disease Clinic Staff, Staff Researcher.

Jul 1982 - Jun 1984 : General Leonard Wood Army Community Hospital, Internal Medicine Staff

Professional Responsibilities: Teaching, Clinical, Administration, Consulting-41%; Research-59%; Total 100%.

Professional Organizations and Certifications:

American Board of Internal Medicine - Internal Medicine Specialty Certification - 15 September 1982.
American Board of Internal Medicine - Subspecialty Certification in Infectious Diseases - 11 November 1986.
American Association for the Advancement of Science., American Society for Microbiology - RNA viruses.
American College of Physicians, International AIDS Society, American Medical Association.
AMA Physicians Recognition Award 1990-1992

State Licensure:

State of Missouri, State of California

Publications:

1. **Looney DJ**, Redfield RR. HTLV Retroviruses and Transfusion Transmitted Diseases. In S.J. Insalaco and J.E. Menitove (Eds.) Transfusion Transmitted Viruses: Epidemiology and Pathology. Arlington, VA:AABB.131- 153(1987).
2. **Looney DJ**, Fisher AG, Putney SD, Shaw GM, Redfield RR, Gallo RC, Wong-Staal F. Type restricted neutralization of molecular clones of HIV. In Girard, M., and Valette, L. (Eds.): Retroviruses of Human AIDS and Related Animal Diseases. Paris, Marnes-la-Coquette, 208-213(1987).
3. Mitsuya H, **Looney DJ**, Kuno S, Ueno R, Wong-Staal F, Broder S. Dextran Sulfate Suppression of Viruses in the HIV Family: Inhibition of Virion Binding to CD4+ Cells. Science 240:646-648(1988).
4. **Looney DJ**, Fisher AG, Putney SD, Rusche JR, Redfield RR, Burke DS, Gallo RC, Wong-Staal F. Type Restricted Neutralization of Molecular Clones of Human Immunodeficiency Virus. Science 241:357-359(1988).
5. Fisher AG, Ensoli B, **Looney DJ**, Rose A, Saag M, Shaw GM, Hahn GH, Wong-Staal F. Biologically diverse molecular variants within a single HIV-1 isolate." Nature 334:444-447(1988).
6. Lee S-J, Hu W, Fisher AG, **Looney DJ**, Kao VF, Mitsuya H, Ratner L, Wong-Staal F. Role of the carboxy-terminal portion of the HIV-1 transmembrane protein in viral transmission and cytopathogenicity. AIDS Res. and Human Retrovir. 5:441-449(1989).
7. Yourno J, Fisher AG, **Looney DJ**, Gallo RC, Wong-Staal F. A recombinant clone of HIV-1 preferentially transmitted in normal peripheral blood mononuclear cells. AIDS Res. and Human Retrovir. 5:565-575(1989).
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9309-058 Economou, James S. and Glasby, John A.; University of California Medical Center, Los Angeles, California; *Genetically Engineered Autologous Tumor Vaccines Producing Interleukin-2 for the Treatment of Metastatic Melanoma.*

Date of RAC Approval: 9/10/1993
Date of NIH Approval: 12/2/1993

ABSTRACT OF PROTOCOL

This protocol is a study of patients with metastatic melanoma who have failed standard therapy. In an attempt to increase the patient's immune response to the tumor, the interleukin-2 (IL-2) gene will be introduced into a human melanoma cell line (M-24). This gene-modified melanoma cell line producing IL-2 will then be mixed with tumor cells obtained from the patient. This mixture of cells will be irradiated to prevent possible growth and injected subcutaneously into the patient. This injection is expected to augment the immune responses of the patient to tumor cells through the immune stimulatory effects of the IL-2 secreted by the gene-modified cells. Animal models have shown the injection of gene modified cells has important antitumor effects. To determine the safety of the procedure, a constant number of tumor cells will be mixed with escalating numbers of IL-2 producing cells.

The patients will be evaluated for antitumor effects engendered by the injection of the gene modified cells mixed with patient tumor cells. The injection of gene modified cells with the tumor cells may serve to "immunize" the patient to their tumor and may be amenable to use in a wide variety of tumor types. This protocol differs from other previous IL-2 "tumor vaccines" in that a wider range of patients and tumor types can potentially be treated as tumor cell lines from each patient do not need to be established.

NONTECHNICAL ABSTRACT OF PROTOCOL

Many patients have advanced cancer that has not responded to standard therapies. For these patients, a new approach to treat their cancer (tumor) will be tested. Animal studies have been done using cancer cells into which a cancer fighting gene has been placed. When these modified cancer cells are returned to the animal, the animal can fight the cancer cells better. One of the cancer fighting genes that can be placed in these cancer cells is a white cell activating factor (interleukin 2). If the gene for interleukin-2 is placed into the tumor cells the tumor cells are made to release interleukin-2. If these gene modified cancer cells are used, the release of interleukin-2 by the cancer cells further increases the ability of the animal's white cells to fight the cancer cells by activating these white cells.

This protocol is designed to copy the results obtained with the animals.
Patients with cancer who have not responded to therapy will have a piece of their cancer cut out and brought to the laboratory. In the laboratory the cancer cells will be mixed with another cell that has been made to release interleukin-2. The patient's cancer cells mixed with the interleukin-2 producing cells are then injected under the skin of the patient. Every few weeks the patient will return to the clinic to receive an additional injection of mixed cells. The amount of interleukin-2 releasing cells will be gradually increased during the period the person receives the injections. During this time and afterwards the patient is watched for signs that the therapy may be activating white cells that could be helpful in destroying cancer cells. Since this therapy is new, it is not known how well it will work in people. The patients will be watched for possible harmful effects from the therapy. After many patients with many different kinds of cancer have this new therapy, the doctors will be able to tell how safe this new therapy is and begin to assess how well it works. The information the doctors gain from this study may permit the doctors to develop better ways of treating patients with cancer.

GENETICALLY-ENGINEERED AUTOLOGOUS TUMOR VACCINES PRODUCING INTERLEUKIN-2 FOR THE TREATMENT OF METASTATIC MELANOMA

UCLA HUMAN GENE THERAPY PROTOCOL #2

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PROTOCOL ROSTER

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PROTOCOL SYNOPSIS

Title :Genetically-Engineered Autologous Tumor Vaccines Producing Interleukin-2 for the Treatment of Metastatic Melanoma

Objective: To evaluate the safety and efficacy of administering a live, irradiated autologous tumor cell mixed with irradiated IL-2 producing melanoma cell line.

To evaluate the immunological and clinical responses of patients receiving the vaccine preparation.

Population: Patients with metastatic melanoma

Sample Size: 10 patients in 3 dose-escalation groups (total of 30 patients)

Dosage/Treatment: 10^7 autologous tumor cells admixed with an allogeneic melanoma cell line (M24-IL-2) transduced with IL-2 gene. Sufficient numbers of M-24 IL-2 will be added to produce 10^3 , 10^4, or 10^5 pg/24hr according to treatment group.

Duration: Patients will be vaccinated biweekly for 6 doses, then monthly thereafter for up to one year.

Endpoints: Safety: local and systemic toxicity

Efficacy: Clinical response of measurable lesions, immunological response to tumor cell skin tests.

Investigators: James S. Economou, M.D., Ph.D.
John A. Glaspy, M.D.

Collaborating Biotechnology Company: Genetic Therapy, Inc., Gaithersburg, MD

PROTOCOL SCHEMATIC

1. Patient screening and Informed Consent

2. Resection of metastatic melanoma with cyropreservation of fresh tumor and attempt to establish cell line.

3. Vaccination in subcutaneous sites biweekly x6 and monthly thereafter for up to 1 year of melanoma vaccine. Vaccine preparation:

Autologous Tumor		M-24 (IL-2)
10^7 cells		10 patients each in
freshly cyropreserved	+	dose-escalation groups
or		A 10^3 pg IL-2/day
melanoma cell line		B 10^4 pg IL-2/day
(only if sufficient fresh		C 10^5 pg IL-2/day
tumor not available)		

4. Treatment Evaluation

   A. Clinical response of measurable lesions

   B. Autologous tumor cell skin test with biopsy (week 0,4,8,12, then monthly for up to 1 year)

   C. Evaluation of peripheral PBL for CTL/LAK activity.

SCIENTIFIC ABSTRACT

This is an open, phase I clinical trial to evaluate the safety and efficacy of using an IL-2 producing autologous tumor cell vaccine preparation in the treatment of advanced melanoma. The vaccine will be composed of irradiated melanoma cell line (M-24) genetically engineered to produce human interleukin-2 (IL-2) and irradiated fresh or cultured autologous tumor cells. Patients will receive biweekly vaccinations,. The following end-points will be measured: 1) toxicity, 2) generation of cytotoxic T lymphocyte (CTL) precursors, lymphokine-activated killer (LAK) cells and enhanced natural killer (NK) activity, 3) immunohistochemistry and delayed-type hypersensitivity of tumor skin tests and 4) clinical response of measurable lesions and/or duration of remission.

NONTECHNICAL ABSTRACT

The immune system has difficulty recognizing and destroying cancer cells. However, if cancer cells are genetically-engineered to produce interleukin-2 (IL-2), an immunostimulating growth factor, they appear to be much more effective in generating an anti-cancer immune response. In animal tumor vaccine experiments, it is not essential that the cancer cells themselves produce IL-2, as long as they are mixed with an IL-2 producing cell.

In this clinical trial, patients with metastatic melanoma will be treated with a vaccine preparation consisting of their own melanoma cells mixed with a tissue culture melanoma line engineered to produce IL-2. The purpose of the IL-2 producing cell line is to provide for the continuous production of the immunostimulating growth factor n the vicinity of the patient's tumor cells. The objective is to have the patient's immune cells recognize their tumor as foreign and generate an anticancer response.

The goals of this trial are to determine if an immune response is generated (using skin tests and lymphatic assays), whether any clinical responses are observed, and what sorts of toxicity are associated with this treatment.

1.0 BACKGROUND AND RATIONALE

Cytotoxic T lymphocytes (CTL) recognize peptide antigens presented on the cell-surface in the context of major histocompatibility complex (MHC) class I molecules. Most CTL responses require T helper cell collaboration which provides for local secretion of cytokines such as interleukin-2 (IL-2). It has been hypothesized that the failure of most cancers to elicit an effective CTL response is, in part, due to a failure of T cell help.

This hypothesis has been tested by providing a continuous supply of the helper cytokine IL-2 at the tumor site. In earlier studies, repeated local tumor injections of IL-2 were employed[1], but with the availability of plasmid or retroviral-based expression vectors, tumor cells can be genetically-engineered to produced IL-2[2-10] . The following principles have emerged from these studies: 1. poorly immunogenic tumors engineered to produce IL-2 have reduced tumorigenicity and can be rejected in vivo, 2, mice acquire protective immunity against parental cells, 3. the response is CD8-dependent, 4. the response is dose-dependent, 5. irradiated IL-2 producing tumor cells generated systemic immunity and 6. syngeneic , allogeneic, xenogeneic cytokine-producing cell lines, admixed with parental tumor effectively abrogates tumorigenicity and generates systemic immunity.

These observations provide the experimental foundation for using genetically-engineered vaccines to generate cell-mediated immunity in human cancer patients. However, there are several logistical problems in generating cytokine gene-transduced autologous tumor cell lines for use as vaccines. The establishment of a tumor cell line for each patient is expensive and labor-intensive and requires the transduction, selection, characterization and biosafety testing for each patient. Some tumor types cannot be adapted to tissue culture. Moreover, tissue culture may result in antigen drift or selection in vitro of nonrepresentative tumor cell populations.

Another approach is to exploit the bystander effect of a cytokine production well documented in animal models. An effective local and systemic immune response can be generated with unmodified tumor cells if they are admixed with cytokine-producing cells of any histological type.

Our approach will be to use a well characterized melanoma cell line (M-24) engineered to produce human recombinant IL-2. The M-24 melanoma cell line has been safely used as an allogeneic melanoma vaccine in hundreds of melanoma patients.[11,12] It has also been used in an FDA and IRB-approved trial in melanoma patients receiving systemic IL-2. (see ref 11 in Appendix II) Vaccines will consist of fresh (or cultured) autologous tumor cells admixed with IL-2 transduced M-24. This approach has several theoretical advantages: (1.) patients receive a well-characterized and biosafety-tested genetically-engineered cell line producing reproducible levels of IL-2, (2.) higher levels of cytokine production can be achieved since higher producer clones are isolated, (3.) allogeneic cell lines may incite a strong local inflammatory response above that generated by IL-2 production, and (4.) if effective, larger numbers of patients with different tumor types could be treated.

2.0 STUDY DESIGN

This is phase I, single site, dose escalating study using fresh or cultured autologous melanoma admixed with IL-2 producing allogeneic melanoma cell line M-24 and administered as an irradiated vaccine. The choice of fresh or cultured autologous tumor cells will depend on the ability to establish a cell line and the amount of tumor resected. Patients will be vaccinated on a biweekly basis for 3 months, then monthly thereafter for up to one year. Eligible patients will have metastatic melanoma. Patients will be followed and studied bi-weekly for evidence of a tumor-specific immunological or clinical response. The groups of 10 patients will receive increasing numbers of IL-2 transduced M-24 with a constant number of tumor cells as a dose escalation to evaluate toxicity and efficacy.

Vaccine composition groups will be as follows:

	Group A	Group B	Group C
Autologous melanoma (fresh tumor or cultured cell lines)	10^7	10^7	10^7
Addition of sufficient numbers of IL-2 transduced M-24 to produce the following amount of IL-2 per 24 hr.	10^3pg	10^4pg	10^5pg
Number of Patients Treated	10	10	10

3.0 STUDY TREATMENT

PREPARATION OF GENETICALLY-ENGINEERED AUTOLOGOUS TUMOR CELL VACCINE

All manipulations will be conducted in the Cellular and Genetic Therapy Core Laboratory in the UCLA Jonsson Comprehensive Cancer Center.

3.1 Preparation of Autologous Tumor

Patient vaccines will be composed of either freshly cryopreserved autologous tumor or tissue culture cell lines established from the patient's tumor.

3.1.1. Preparation of Tumor Cells

Solid tumors will be resected in the operating room, minced into 5 mm^3 pieces and placed in an enzymatic digestion medium composed of RPMI 1640 (Flow Laboratories), containing hyaluronidase type V (0.01%), DNase I (0.002%), collagenase type IV (0.01%). (Sigma, St. Louis, MO), penicillin (50 IU/ml),

streptomycin (50 μg/ml) (Flow) and gentamicin (50 μg/ml) (Gibco) Enzymatic digestion is conducted at room temperature with stirring for 18 hrs. The tumor single cell suspension is washed 5x in Hanks Balanced Salt Solution.

3.1.2. Establishment of Tumor Cell Line

An attempt to establish a tumor cell lines in tissue culture for each melanoma biopsied. The tumor cell suspension will be placed in RPMI 1640, 10% heated-inactivated pooled human AB serum and antibiotics at a concentration of $5x10^5$ viable tumor cells/ml and cultured in a 5% CO_2 incubator. Successfully established cell lines will be characterized by histology, immunohistochemistry (when indicated), and tested for bacterial, fungal or mycoplasma prior to use.

In all instances, freshly isolated tumor cells will be cryopreserved as described below in 6.1.3. These frozen tumor cells will be needed for skin testing and, if sufficient numbers are available, as vaccine immunogen, if a cell line cannot be established.

3.1.3. Cryopreservation of Fresh Autologous Tumor

The single cell suspension will be cryopreserved at-70^0c in 90% heat-inactivated autologous serum, 10% DMSO at a final concentration of 10^7 tumor cells/ml. Autologous serum will be prepared from the pretreatment blood draw.

3.2 Human IL-2-Producing M-24

M-24 cells transduced with an IL-2 retroviral vector (G1Na CV IL-2), selected in G418, and cloned for high production will be used as the IL-2 producing cell line to be included in the tumor cell vaccine. This replication-defective retroviral vector will be provided by Genetic Therapy, Inc., Gaithersburg, MD. This cell line M-24 (IL-2) will be maintained in the Cellular and Genetic Therapy Core Laboratory on the 14th floor of the Factor Building in the Jonsson Cancer Center. A large master cell bank of sufficient numbers of cells to complete this protocol will be prepared and cryopreserved in 90% fetal bovine serum and 10% DMSO. This cell bank will pass the following tests:

a. hIL-2 production by ELISA assay (not less than 10^4 pg/10^6 cells/24 hr)
b. Absence of aerobic, anaerobic, fungal and mycoplasma contamination
c. Free of replication competent retrovirus using S+L-assay with 3T3 amplification
d. Viability > 95%

3.2.1. Preparation of M-24 (IL-2)

On the day of vaccine preparation, an aliquot of M-24 (IL-2) will be thawed, washed 5x in Hank's Balance Salt Solution and adjusted to the appropriate numbers to produce either 10^3, 10^4, or 10^5 pg/24 hr IL-2 depending on the vaccine treatment group.

3.3 **Vaccine Preparation**

Autologous tumor cells (10^7) and an appropriate number of M-24 (IL-2) will be admixed, resuspended in 0.5-1.0 ml HBSS and irradiated to 5000 cGy in a cobalt 60 Gamma Cell Irradiator (AEC).

3.4 Vaccine Administration

The irradiated vaccine will be injected subcutaneously in two subcutaneous deposits on either flank in the mid axillary line. The vaccination site will be marked with a small India ink tatoo.

Two hours prior to each vaccine administration, patients will receive an intradermal injection of 10^3 irradiated (5000 cGy), untransduced M-24 in a volume of 0.05 ml in saline. The skin wheal and erythema will be compared with a positive histamine control.

4.0 PATIENT SELECTION

4.1 Inclusion Criteria

4.1.1 This study is confined to adults over the age of 18.

4.1.2 Both male and female patients may be enrolled. All patients should be counselled regarding the need for effective contraception. Premenopausal females must have a negative pregnancy test prior to treatment.

4.1.3 Patients must be ambulatory with a Karnofsky Performance Status greater than or equal to 70 percent.

4.1.4 Patients must have no evidence of class 3 or greater New York Heart Association cardiac insufficiency or coronary artery disease.

4.1.5 No evidence of opportunistic infection.

4.1.6 A minimum of 4 weeks must have elapsed since the completion of prior chemotherapy or radiation therapy.

4.1.7 Adequate baseline organ function as assessed by the following laboratory values within 30 days prior to study entry (day -30 to 0):

- Adequate renal function with estimated creatinine clearance > 50 ml/min. This will be determined by serum creatinine and 24-hour urine creatinine ordered concurrently.

- Adequate liver function with SGOT, SGPT and alkaline phosphatase $\leq$ 5 times the ULN [if transaminases > than the upper limit of normal (ULN), patients should have a hepatitis B

surface antigen (HBsAG) test prior to study entry. Patients may not enter the study if HBsAG is positive.]

- PT and PTT not more than 1.5 times the ULN
- Hemoglobin $\geq$ 9.0 g/dl (patients cannot be transfusion dependent)
- Platelets $\geq$ 75,000/mm^3
- WBC > 2,000/mm^3
- Absolute Neutrophil Count (ANC) $\geq$ 1,000/mm^3
- Serum Amylase $\leq$ 1.5 times the ULN
- Bilirubin $\leq$ 2.5 mg/dL

4.1.8 Ability to give informed consent

4.1.9 Normal cardiac function by history and exam

4.1.10 Karnofsky Performance Status $\geq$ 40

4.2 Exclusion Criteria

Patients who meet any one of the following criteria will be excluded from study entry:

4.2.1 Pregnant or lactating females [all patients must practice adequate birth control and females of child-bearing potential must have a negative serum beta-HCG pregnancy test (within Day -7 to Day 0)].

4.2.2 Acute infection: any acute viral, bacterial, or fungal infection which requires specific therapy. Acute therapy must have been completed within 14 days prior to study treatment.

4.2.3 Hepatitis-B surface antigen positive patients.

4.2.4 HIV-infected patients.

4.2.5 Acute medical problems such as ischemic heart or lung disease that may be considered an unacceptable anesthetic or operative risk.

4.2.6 Patients with any underlying conditions which would contraindicate therapy with study treatment (or allergies to reagents used in this study).

4.2.7 Patients with organ allografts

4.3 Concomitant Medication and Treatment

All allowed medications or treatments should be kept to a minimum and recorded. All questions regarding concomitant medications should be referred to the study chair or investigator.

4.4 Medications and Treatments Not Allowed

Corticosteroids

5.0 CLINICAL AND LABORATORY EVALUATIONS

5.1 Screening and Baseline Evaluations

The following clinical and laboratory evaluations will occur within Study Days -30 to 0 unless otherwise noted. Screening evaluations, or evaluations that determine patient eligibility, are noted. These screening evaluations must be reviewed prior to study treatment.

5.1.1 Objective Signs and Symptoms: include vital signs (blood pressure, pulse, temperature and respirations) weight and height. (Screening and repeat Day 0)

5.1.2 History and Physical Examination: Screening, repeat targeted physical exam on Day 0

5.1.3 Karnofsky Performance Status (see Appendix IV) (Screening)

5.1.4 Chest X-Ray: Normal CXR within the last 30 days unless new symptoms suggest that this be repeated prior to dosing (Screening)

5.1.5 Electrocardiogram (ECG): ECG must be normal within 30 days unless new symptoms suggest this be repeated prior to dosing (Screening)

5.1.6 Urinalysis: Routine urinalysis including a 24-hour creatinine clearance (Screening and repeat Day 0)

5.1.7 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase, triglycerides, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, and glucose (Screening)

5.1.8 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen (Screening).

5.1.9 Pregnancy Test: Serum pregnancy test must be negative within 7 days prior to Day 0, or between study days -7 and 0.

5.2 Interval Evaluations

Evaluations are done on a monthly basis.

5.2.1 Objective signs and symptoms: Vital signs (blood pressure, pulse, temperature and respirations) weight and height will be done.

5.2.2 Physical Examination: Target physical exam will be done on Study Days.

5.2.3 Karnofsky Performance Status (see Appendix IV) will be done Study Days.

5.2.4 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase, triglycerides, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, and glucose.

5.2.5 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen.

5.2.6 Pregnancy Test: Serum pregnancy test will be repeated monthly.

5.3 Post-Treatment Evaluations

5.3.1 The same evaluations as section 5.2 (except 5.2.5 and 5.2.6) will be performed bimonthly.

5.3.2 **Autopsy Request**: All participants in the study will be informed that an autopsy will be requested to determine the extent of gene transfer into important organs such as marrow, liver, and spleen.

6.0 TREATMENT MODIFICATION AND DISCONTINUATION ACCORDING TO LEVEL OF TOXICITY

The GTI Table for Grading Severity of Adverse Experience (Appendix II) will be used to achieve consistency in response to drug/treatment toxicities. Toxicities will be graded on a 1 - 4 grading scale. If a toxicity is experienced, the treatment level or dose will be modified (if applicable) as outlined below according the grade of toxicity observed

6.1 Treatment Modification and General Management of Toxicities

- For any Grade 1 toxicity, there will be no dose modification.
- If a Grade 2 toxicity develops, the investigator may elect to continue the treatment with careful monitoring or to hold the (drug) until values return to Grade 1 or less, then restart the drug.
- In general, if a Grade 3 or 4 toxicity is obtained, the (drugs) will be held until a the Grade reaches $\leq$ 1. Repeat tests to confirm values within 72 hours will be required.
- Any Grade 3 toxicity, thought to be related to IL-2 will necessitate a 50% dosage reduction. If Grade 3 toxicity persists for seven days or advances to grade 4 toxicity, IL-2 dose will be reduced to 10%.
- For Grade 3 toxicity not related to IL-2, the investigators and collaborators will discuss the various options available. The appropriate action relative to the patient with a Grade 3 toxicity as well as a discussion of enrolling other patients will be addressed. Since no toxicities have been seen in other gene transfer protocols, precedence for the appropriate action is not available and will need to be decided on a case-by-case basis.

6.2 Symptomatic Therapy for Toxicity

Symptomatic therapy such as analgesics or other helpful therapy may be administered if deemed necessary by the participating investigators. All medications administered will be recorded in the appropriate Case Report Form.

7.0 CRITERIA FOR DISCONTINUATION OF STUDY PATIENTS

7.1 Criteria for Treatment Discontinuation

Participation in this study can be discontinued for any of the following reasons listed below:

7.1.1 At the Patient's request: Any patient who wishes to withdraw from the study may do so but will be counselled that long-term follow-up for the effects of gene therapy are strongly desired by the medical facility and federal regulatory groups.

7.1.2 A major, unexpected, or life-threatening toxicity requiring that treatment be discontinued

7.1.3 Generalized impairment or mental incompetence which would relinquish the patient unable to understand his/her participation in the study.

7.1.4 If, in the Investigator's medical judgment, further participation would be injurious to the subject's health or well-being.

7.1.5 Administrative reasons, such as subject non-compliance or a major protocol violation.

7.1.6 Pregnancy

An explanation will be recorded for any patient that is taken off treatment and the appropriate Case Report Form completed.

7.2 Criteria for Study Discontinuation

Patients should understand that once they have received one vaccination, they will be asked to be followed long-term. They can stop study treatment at any time (see section 7.1), however they will still be considered on study until the study has been terminated.

The study can be discontinued only with the understanding of the investigators, sponsor, RAC and the FDA.

8.0 ADVERSE EXPERIENCES

Appendix III is Adverse Experience Reporting

Depending on the extent of the adverse experience, adverse events should be reported by the investigator to their local Institutional Review Board (IRB), Institutional BioSafety Committee (IBC) and to Genetic Therapy, Inc. (collaborating company) as specified in Appendix III.

Adverse reactions or deaths requiring immediate reporting should be made by telephone to (301) 590 2626 (available 24 hours a day, recorder after working hours).

The sponsor, Genetic Therapy, Inc. will take the responsibility of informing and preparing reports for the Recombinant Advisory Committee (RAC) through the NIH Office of Recombinant DNA Activities (ORDA) and to the FDA.

HHS Guidelines for Research Involving Recombinant DNA Molecules: It is the responsibility of the institution and those associated with it to adhere to the intent of the *Guidelines* as well as to their specifics.

9.0 EVALUATION OF RESPONSE/ENDPOINTS

9.1 Toxicity

Toxicity is described in 6.1 and Appendix II. The MTD (maximum tolerated dose) is defined as that dosage level immediately below the dosage level at which 2 patients of a cohort experience dose-limiting toxicity.

9.2 Clinical Response

Patients with measurable disease will have serial measurement of such indicator lesions which will be considered for response according to the definitions below, at 6 weeks, and thereafter.

9.2.1 Complete Response (CR): Total disappearance of all measurable disease, M-1 marrow status with restoration of normal hematopoiesis and normal performance status persisting at least 4 weeks. Onset of response will be appropriately noted and detailed, and duration noted.

9.2.2 Very Good Partial Response (VGPR): Regression of primary tumor (>90%), clearing metastatic disease except for persistent bone scan positive lesions attained by and maintained through induction.

9.2.3 Partial Response (PR): Regression of >50% of all tumor.

9.2.4 Stable Disease: Exists when a patient fails to qualify for either complete response, VGPR, partial response or progressive disease.

9.2.5 Progressive Disease: Worsening of disease, evidenced by enlargement of existing tumors or appearance of new disease.

9.3 Immunological End-Points

The principal evaluable goals of this phase I trial are largely immunological and include skin tests, evaluation of lymphocyte cytotoxicity, measurement of plasma cytokine levels and immunohistochemical analysis of tumor biopsies.

9.3.1 Skin tests

Patients will be skin tested with an intradermal injection of irradiated (5,000 c Gy), cryopreserved autologous tumor. The skin test will be given prior to treatment and at monthly intervals after treatment initiation. The skin test dose will be $5x10^5$ cryopreserved cells in 0.05 ml normal saline injection intradermally. Erythema and induration will be measured in millimeters 24 and 48 hr after injection. The skin test site will be on either arm.

Up to 14 days later, punch biopsies (3mm) will be obtained of the skin test after local infiltration with 1% lidocaine. The punch biopsies will be evaluated to establish the nature of the cellular infiltrate. Tissue samples will be processed for immunohistology and stained with antisera to detect infiltrating T cells (CD4, CD8), B cells (CD20), NK cells (CD56) and macrophages (CD14, CD15). These will be graded by an independent pathologist.

9.3.2. Cytotoxic Lymphocytes

Peripheral blood leukocytes (PBL) will be isolated from heparinized whole blood by Ficoll-Hypaque centrifugation. A portion of these cells will be immediately tested for cytotoxicity against a battery of targets - K562 (an NK-sensitive cell line), M-14 (an NK-insensitive melanoma cell line) and autologous tumor (if a cell line or cyropreserved targets are available). - using a standard 4 hr. chromium-response assay. Another aliquot of cells will be cultured in the presence of 40 u/ml IL-2 for 3-5 days and the same cytotoxicity testing performed.

9.3.3. Tumor Biopsies

Patients with subcutaneous disease will be asked to consent to have punch biopsies of tumor deposits. These biopsies will be performed prior to and at monthly intervals after initiation of treatment. The biopsies will be processed using the same immunohistochemical stains described in 9.3.1.

9.3.4. Plasma Cytokine Levels

The plasma from each blood draw will be stored at - 70°C. IL-2 levels will be measured using commercially available ELISA plates (R&D Systems, Minneapolis, MN).

10.0 DOCUMENTATION, RECORD KEEPING, CRFs

The investigator will maintain adequate records so that the conduct of the study can be fully documented and monitored.

Copies of protocols, case report forms (CRFs), test result originals, all product accountability records, correspondence, patient informed consent, and any other documents relevant to the conduct of the study will be kept on file by the investigator for (five) years after all investigational use of product is discontinued and the FDA is so notified or until five years after a Product Licensing Application (PLA/ELA) is approved. Study documents will not be destroyed. For FDA inspections, will be necessary to have access to complete study patient records, provided that patient confidentially is maintained.

The investigator will obtain a separate release of medical information form to be signed by the study patient in order to facilitate access to the patient's medical records should

the patient be hospitalized at an institution with which the study investigator is not associated.

A record will be kept of all patients considered (and screened) for the study and subsequently deemed ineligible. The reason for ineligibility must be recorded.

11.0 DATA COLLECTION AND STUDY MONITORING

11.1 Data Collection

Case Report Forms (CRFs) will be utilized for each patient entered into the study. Study participants will NOT be identified by name on any study documents. Patients will be identified by a patient identification number (PIN). Investigators will need to keep a patient code list accessible.

12.0 BIOSTATISTICAL CONSIDERATIONS

The end-points of this study are toxicity, immunological response (cytoxicity, skin test immunohistology) and clinical response.

Patients will serve as their own controls. Pre - and post-treatment values will be generated as stem and leaf plots. Statistical tests will be applied to these paired data with appropriate confidence intervals and the ability to carry out multiple tests.

13.0 ETHICAL CONSIDERATIONS

The investigator will ensure that the study is conducted in full conformance with the FDA standards for human research.

13.1 Informed Consent

All study participants must sign an informed consent (see Appendix VI). The investigators will inform all subjects as to the nature, aims, duration, potential hazards, and procedures to be performed during the study and that his or her medical records may be reviewed by the sponsors or the FDA. This protocol must receive approval by the Institutional Review Board prior to implementation. The investigator must also explain that the patients are completely free to refuse to enter the study or to withdraw from it at any time. The protocol will be discussed in detail with all potentially eligible patients. All revisions of the protocol must be reflected in the consent form and reviewed by the IRB.

13.2 Patient Confidentiality

All reports and patient samples will be identified only by a coded number to maintain patient confidentiality. All records will be kept confidential to the extent permitted by law. The investigator should keep a separate log of patients' codes, names, and addresses. Documents which identify the patient by name (informed consent) should be kept in strict confidence.

3.3 Patient's Financial Responsibilities During the Study

A grossly exaggerated upper limit is outlined in the Informed Consent Form at the request of the UCLA IRB.

14.0 CRITERIA AND PROCEDURES FOR PROTOCOL MODIFICATION AND STUDY TERMINATION

Modifications which may effect the safety of the study patient, or which may alter the scope of the investigation, the scientific quality of the study, the study design, dosages, duration of therapy, patient assessments (added evaluation that poses potential risk or inconvenience to the patient), number of patients, and patient eligibility criteria, may be made only after appropriate consultation between the sponsor and the investigator.

If the consensus is to revise the current protocol, a formal List of Changes will accompany the amended protocol and these will be submitted to the FDA, the site's IRB, and other committees as required.

Both the sponsor and the investigator reserve the right to terminate the study at any time. If this becomes necessary, appropriate procedures for continuing the long-term follow-up requested by the regulatory agencies will be arranged after review and approval by both parties.

15.0 REFERENCES

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UNIVERSITY OF CALIFORNIA, LOS ANGELES
CONSENT TO ACT AS A SUBJECT IN A RESEARCH PROJECT

TITLE: Genetically-Engineered Autologous Tumor Vaccines Producing Interleukin-2 for the Treatment of Metastatic Melanoma

INVESTIGATORS: James S. Economou, M.D., Ph.D., John Glaspy, M.D., Jane Hobbs, R.N., Phillip Koeffler, M.D., William H. McBride, Ph.D., ScD., Robert Moen, M.D., Ph.D., Joseph Rosenblatt, M.D., Gary Schiller, M.D., Hungyi Shau, Ph.D., Dennis Slamon, M.D., Ph.D.

1._____ _____
Patient's Name UCLA ID Number

I have the right to know about the procedures that are to be used in my participation in clinical research to afford me an opportunity to make the decision whether or not to undergo the procedure after knowing the risks and hazards involved. This disclosure is not meant to frighten or alarm me; it is simply an effort to make me better informed so I may give or withhold my consent to participate in clinical research. This informed consent does not supersede other informed consents I may have signed. I understand that I have been asked to participate in this study because I have a type of cancer, malignant melanoma, that is unlikely to respond to conventional therapy or one that has stopped responding to such therapy.

Description or Research

2. Purpose of study: I understand that this is a research study to treat me with an experimental preparation composed of a mixture of cells from my own tumor and a melanoma cell line, originally derived from a patient with melanoma, that has been genetically altered to produce interleukin-2, a hormone that stimulates the immune response. I am aware that the experimental preparation I am being asked to consider is unproven and that this is a Phase I Trial designed to evaluate its safety as much as its effectiveness.

Before beginning treatment, I understand that I will undergo the following studies: a history and physical examination, an appropriate radiographic study (chest x-ray, Computed Tomogram or Magnetic Resonance Imaging scans), electrocardiogram, urinalysis, and blood tests which will require 6 tablespoons of blood removed by needle puncture of a vein. (The schedule and number of tests are on page 5.) During the treatment/test period, up to 11 blood-draws of 6 tablespoons of blood may be used. In order to prepare the vaccine, I understand that one or more subcutaneous (under the skin) tumors will have to be surgically removed from my body. I understand that I will receive separate, detailed, informed consent for this surgical procedure. This tumor will be used to prepare an experimental vaccine. I understand that an effort will be made to culture my tumor so that it can be grown into large numbers and that this cell line of my tumor may be used in the experimental vaccine preparation. The purpose of this vaccine is to stimulate my immune system to fight my cancer. It may require weeks or months to establish a cell line.

The vaccine given to me will also consist of a melanoma cell line called "M-24" that has been genetically modified to produce interleukin-2. The interleukin-2 gene has been inserted into these cells using a mouse virus called a retrovirus. This inserted gene allows the melanoma cells to produce interleukin-2. Interleukin-2, is a hormone which stimulates the immune response.

It is hoped that IL-2 production by cells mixed with my cancer cells will provoke an immune response against my tumor.

I understand that my tumor cells and the melanoma cell line producing interleukin-2 will be mixed and injected into my skin using a small needle in up to four sites on either side of my body. These cells will receive x-ray treatment prior to vaccination to prevent the possible growth of my cancer cells at the injection site. Injection sites may be marked with a small spot of India Ink. My vaccination dose may be composed of different numbers of M-24 melanoma cells to test the effects of different doses of interleukin-2. I understand that I may receive vaccinations on weeks 0, 2, 4, 6, 8, 10, 12 and monthly thereafter for up to one year. Fewer treatments may be recommended if there are insufficient tumor cells or if I experience untoward side-effects.

In an effort to understand if the vaccine is stimulating my immune system, I understand that several tests will be performed. If I have cancer deposits underneath my skin, I will have pretreatment and post-treatment pieces of tumor removed under local anesthesia using a 6 mm punch biopsy knife (no more than 5 biopsies at intervals no less than one month). These biopsies will not involve a cost to me. I may also be asked to receive a monthly skin test injection that will consist of my irradiated tumor cells without the M-24 melanoma cells cell line to monitor any immune response. These skin test sites may also be biopsied under local anesthesia using a 6 mm punch biopsy knife but I will be asked to submit to no more than 5 of these skin test biopsies. I understand that all biopsy procedures will require individual surgical consents and that I may refuse any without prejudice.

RISKS, SIDE EFFECTS AND DISCOMFORT FOR PARTICIPANTS

I know that at least one operation will be required to obtain enough tumor for vaccine preparation or culture. I will receive a separate informed surgical consent for this operation. There are some risks associated with the punch biopsies of tumor or skin test sites and include pain, bleeding and infection which are easily treated.

I understand that the risks associated with vaccination include local pain, inflammation and possible infection at the injection site. Interleukin-2 production can cause tiredness, fever, chills, sweats, nausea, vomiting, shortness of breath, nasal congestion, confusion, seizures, low blood pressure, low urine output, heart attack, arthritis, vision changes, low blood counts, muscle pains, knee tenderness, infection, flu-like symptoms, unknown side-effects, migration of cancer cells in the experimental vaccine and death. Some of these side-effects, if they occur, may be treated by certain medications. Since this trial is designed to evaluate toxicity, all of my organ systems will be closely monitored for side-effects from treatment.

Drawing of blood by vein may be associated with the complications of swelling, pain, bruising and rarely inflammation or infection.

There is a very small chance that this treatment may cause a new cancer. I understand that the vaccine preparation will be irradiated and should be incapable of growth. The M-24 melanoma cells modified to produce interleukin-2 has the remote possibility of producing an infectious virus that could infect other cells in my body and cause a new cancer. There is also the possibility that an immune response may be generated that attacks a normal organ of my body. Because of the possibility of an allergic response to the melanoma cell line, I understand that I will have a skin test prior to each vaccination.

If I could potentially bear a child, I will not participate in this research protocol unless, with the investigator's knowledge and approval, I am employing a medically acceptable form of birth control (contraception). This research may have unforeseeable risks to an embryo or fetus. A pregnancy screening test will be performed at no cost to me and a negative result will be required prior to the initiation of therapy.

POTENTIAL BENEFITS

I understand that there may be no direct benefit to me from study participation, and there is a possibility my condition may become worse. However, a better understanding of immunity to cancer may be gained by my participation that would benefit society in general.

ALTERNATE PROCEDURES OR TREATMENT

This experimental trial is offered to me because my cancer is advanced and alternate methods of therapy - surgery, chemotherapy and/or radiation therapy - have not proven beneficial or are still being tested.

UNDERSTANDING OF PARTICIPANTS

I have been given an opportunity to ask any questions concerning the investigational cancer treatment regimen involved and the investigator has been willing to reply to my inquiries. The Il-2 producing M-24 melanoma cells and my own melanoma cells will be administered under the above numbers, title and described clinical research protocol at this institution. I hereby authorize Dr. James S. Economou and my attending physician/investigator and designated physician associates to administer the investigational cancer treatment for the purpose of determining its side-effects and effect on my immune system.

I have been told and understand that my participation in this clinical research study is voluntary. I may decide not to participate, or withdraw my consent and discontinue my participation at any time. Such action will be without prejudice and there shall be no penalty or loss of benefits to which I may otherwise be entitled, and I will continue to receive treatment by my physician at this institution. I understand that circumstances may arise which might cause the investigator to terminate my participation before completion of the study.

If I decide not to participate or withdraw my consent from participation in this clinical research, I understand that I should discuss the potential consequences or effects of my decision with my physician.

I will be informed of any new findings developed during the course of this clinical research study which may relate to my willingness to continue in the study.

I understand that the Food and Drug Administration and Genetic Therapy, Inc., a collaborating drug company, may inspect records relating to my participation in this study. Therefore, my identity will be known to those agencies and company. I understand that no information which identifies me will be released without my separate consent as specifically required by law.

I understand that I or my insurance company may be charged for any procedure or test that is medically indicated. I may discuss this and ask any questions regarding billing before I consent to treatment. Neither the vaccine nor any tests or procedures that are of an investigative nature will be my financial responsibility.

I understand that if I am injured as a direct result of research procedures not done primarily for my own benefit, I will receive medical treatment at no cost. The University of California does not provide any other form of compensation for injury.

I understand that cells obtained from my tumor may be used to establish a cell line which may be shared in the future with other researchers and which may be of commercial value. A cell line is one which will grow indefinitely in the laboratory. Cell lines may be useful because of the characteristics of the cells and/or products they may produce.

I understand that the possible benefit of this study is the stimulation of the immune response and potential shrinkage of my tumor. Although these treatments may be helpful, these are experimental treatments and therefore it is not possible to determine the likelihood of benefit at this time. I understand that my tumor may progress and my condition may become worse in spite of receiving this experimental treatment.

I understand that I will be followed for life by the Protocol Chairman Dr. James S. Economou. I also understand that shoud I die, a postmortem examination will be requested from my family.

I understand that if I have further questions, comments or concerns about the study or the informed consent process, I may write or call the Office of the Vice Chancellor-Research Programs, 3134 Murphy Hall, UCLA, Los Angeles, CA 90024, (310) 825-8714.

The study and its procedures have been explained to me by _____.
I understand that if I have any questions, I may contact Dr. James Economou (310-825-2644) in the Department of Surgery, UCLA Medical Center. He will answer any questions at any time regarding details of this study. If the study design or the use of the information is to be changed. I will be so informed and my consent will be reobtained.

In signing this form, I acknowledge receipt of a copy of the form as well as a copy of the Subject's Bill of Rights.

TREATMENT AND TEST SCHEDULE

WEEK	M-24 SKIN TEST and VACCINATION	TUMOR CELL SKIN TEST	BLOOD DRAW.[A]	T U M O R BIOPSY[B]
Pretreatment		X	X	X
0	X			
2	X			
4	X	X	X	X
6	X			
8	X	X	X	X
10	X			
12	X	X	X	X
Monthly for up to one year	X	X	X	X

a. 25 ml for cytotoxic lymphocyte tests (up to 12 blood draws may be needed over the course of the treatment/test schedule)

b. Only in patients with subcutaneous disease

_____ _____

Date Signature of Patient

_____ _____

Time Signature of Witness

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person

NAME	POSITION TITLE
James S. Economou, M.D.,	Associate Professor of Surgery

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
The Johns Hopkins University	B.A.	1972	Biology
The Johns Hopkins University	M.D.	1980	Medicine
School of Medicine	Ph.D.	1980	Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1980-1986	Residency in General Surgery	UCLA, UCSF
1986-1990	Assistant Professor of Surgery	UCLA
1990-present	Associate Professor of Surgery	UCLA

Economou JS, Essner R, Rhoades K, McBride WH, Golub S, Holmes EC, Morton DL. Molecular mechanisms of interleukin-2 (IL-2) induction of human necrosis factor (TNF) gene expression in macrophages. Surgical Forum 39:426-428, 1988.

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Chandler, C.F., Skotzko, M.,Lee, J.D., Rhoades, K., Tso, Cho Lea, Belldegrun, A., McBride, W.H., Kohn, D., Golub, S.H., Holmes, E.C., Economou, J.S. Retroviral-mediated gene transfer of interleukin-6 and interleukin-7 into human tumor-infiltrating lymphocytes. Surgical Forum. 42:470-472, 1991.

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person

NAME	POSITION TITLE
John A. Glaspy, M.D.	Assistant Professor of Medicine

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Santa Clara	B.S.	1975	Biology
UCLA School of Medicine	M.D.	1979	Medicine
UCLA School of Public Health	M.P.H.	1979	Health Service Administration

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE

1979-1980	Internship, Internal Medicine, UCLA Hospital
1980-1982	Residency Internal Medicine, UCLA Hospital
1982-1985	Fellowship, Hematology Oncology, UCLA Hospital
1983-1984	Chief Resident in Medicine, UCLA Hospital
984-Present	Assistant Professor of Medicine, UCLA School of Medicine
1985-1989	Medical Director, UCLA Inpatient Solid Tumor Unit
1989-Present	Director, Joint Bowyer/Surgical Oncology Center, UCLA
1990-Present	Associate Chief, Division of Hematology-Oncology, UCLA Department of Medicine; Associate Director, Medical Oncology Program Area, Jonsson Comprehensive Cancer Center

HONORS

1978	Alpha Omega Alpha
1979	Stafford Warren Award for Academic Achievement
1984	UpJohn Award for Excellence in Medicine
1989	Richard & Eleanor Dwyer Fund for Excellence - Annual Cancer Research Award
1991	California Legislature Commendation for establishing the UCLA/Kern Oncology Program

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SFV VECTOR EXPRESSION SYSTEM - G. F. TEMPLE

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July 15, 1993

Nelson A. Wivel, M.D.
Office of Recombinant DNA Activities
National Institutes of Health, 31-4B11
Bethesda, MD 20892

Dear Dr. Wivel:

I am enclosing for your Committee's review a proposed version of a Users' Manual that includes the information requested by RAC, on June 8, in response to our request to classify the Life Technologies SFV/Helper 2 Gene Expression System for use at Biosafety Level 2.

In addition to information presented previously, this manual contains the following:

A summary of potential health risks associated with use of the SFV/Helper 2 Gene Expression System (Section IB);

A description of several practical and effective means to inactivate SFV present in liquid or on surfaces (Section IIIB), together with the experimental investigations and results that support the use of these methods (Appendix C);

A simple protocol for detecting replication-competent SFV present in solutions or cell cultures (Section IIIC);

A description of symptoms associated with SFV infection and procedures to be followed in the event that SFV infection occurs in a laboratory worker, including precautions to prevent interpersonal spread of SFV by mosquitos (Section IC);

Warnings to abide by Biosafety Level 2 guidelines prominently posted on each page of those protocols in Section IIIA which might expose the worker to infectious virus--assuming that RAC classifies this vector system for use at BL 2;

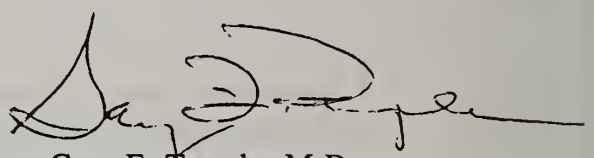
A Biosafety Clearance Agreement that must be signed by the user and his or her Institutional Biosafety Committee and received at Life Technologies, before the SFV/Helper 2 Gene Expression System will be shipped to a customer (Section IA);

A listing of Biosafety Level 2 practices, containment equipment, and laboratory facilities (Section ID), and practices specific for the use of the SFV/Helper 2 Gene Expression System (Section IE).

Please let me know if I can be of further assistance.

Thank you for your consideration.

Sincerely yours,



Gary F. Temple, M.D.
Director, Corporate Research

APPENDIX: Evidence presented by Life Technologies to NIH RAC regarding the biosafety of SFV and derivative vector systems.

A. Biosafety of the SFV/Helper 2 Gene Expression System

1. RATIONALE:

In the experiments described below we examine first the effectiveness of employing helper vectors as a means to prevent emergence of replication-competent virus. Using a model system consisting of SFV3 RNA plus Helper 1 RNA (see p. 22), we measure the frequency of recombinants between helper RNA and vector RNA subgenomes. A low level of replication-competent virus is found.

Therefore a second biosafeguard is created, based on mutations that block the processing of coat proteins needed for virus infectivity. To evaluate the effectiveness of this safeguard, a model vector is constructed wherein mutations are introduced into the genes encoding SFV coat proteins in a replication-competent SFV. Our analysis of this SFV variant, SFV-SQL, reveals a very low level of infectious revertants.

Finally, both biosafeguards are combined into one system: Incorporating the SQL mutation into Helper 1 yields the SFV/Helper 2 expression system. When this dual system is analyzed, both in tissue culture and in animals, no replication-competent virus is detected.

2. RECOMBINATION BETWEEN HELPER 1 AND PSFV3-LACZ RNAS

Use of helper-dependent packaging is an effective means to control the production of infectious virus. This safeguard could be subverted, however, if the helper vector and recombinant vector (carrying the sequences desired to be expressed) were to recombine to restore an intact viral genome. In fact, wild-type recombinant alphavirus genomes can be generated from double infections with two defective genomes (Weiss and Schlessinger, 1991).

It was of interest, therefore, to analyze whether this also applies to the SFV *in vivo* packaging system. Recombinant virus stocks comprising SFV3-lacZ and Helper 1 were analyzed by indirect immunofluorescence screening of infected cells for the occurrence of E2 expression, using a polyclonal antibody directed against E2 protein. E2 protein would be expressed in these cells only if a recombination event had occurred between the recombinant and helper genomes, or if both RNAs had been packaged into the same virus particle. A double infection by recombinant and helper virus particles was minimized by using a low multiplicity of infection.

When a total of 10^7 cells was screened, using two different stocks of SFV3-lacZ (MOI = 0.1), no wild-type particles were found, suggesting that recombination and/or copackaging had occurred in fewer than 1 in 10^6 first-generation virus particles.

Because only a limited number of cells (and virus particles) can be analyzed in this fashion, another strategy was employed to detect low levels of replication-competent virus. SFV3-lacZ/Helper 1 packaged virus stocks were used to infect BHK cells ($1x10^6$) at MOI of 1-10, and infection allowed to proceed for up to 24 hours (Table 1). At 9 hours post-infection, the medium was collected and titrated for plaque-forming units (PFU), evidence of replication-competent virus. From a total of four individual experiments, 70 to 1000 PFU were detected, indicating that a low level of recombination ($\leq 10^{-6}$) had occurred between the helper and SFV3-lacZ RNAs.

Table 1

Production of Replication-Competent Virus from Packaged Recombinant Virus

	Plaque-forming Units[1]	
	Helper 1	Helper 2 (SQL)
Unactivated	70-1000	0,0[2]
Activated	ND[4]	0,0[3]

[1]Numbers of replication-proficient virions, detected as plaque-forming units (PFU), by infecting 10^6 BHK cells with packaged recombinant viruses, at MOI of 1-10, with or without protease activation. Helper 1 was assayed after 9 hours of culture; Helper 2 after both 9 hours and 24 hours, corresponding to the two values given.

[2]Cytopathic effects were totally absent.

[3]All cells demonstrated active viral replication (CPE) and eventual cell death.

[4]ND: Not Determined. Helper 1 virus is infectious without activation.

To obtain a more precise measure of the frequency of recombinants formed from SFV3-lacZ and Helper 1 RNAs under standard packaging conditions, the following experiments were performed. Confluent plates of BHK cells ($2.5x10^6$ cells per plate) were infected with a stock of pSFV3-lacZ/Helper 1 packaged virus, using 10^4, 10^5 or 10^6 particles per plate. In all, sixteen different virus stocks were evaluated. All tests were done in duplicate for each stock. After one hour, the cells were overlayered with agarose (dissolved in complete medium) and incubated for two days, after which the plates were stained for plaques. The

results are displayed in Table 2, showing the mean value for duplicate plates. Based on the observed PFU with 10^5 IU per plate, recombinants appeared, on average, with a frequency of about 4×10^{-4}.

Table 2

Frequency of Replication-competent Virus in Stocks of Packaged SFV3-lacZ/Helper 1

Titre of Stock IU/ml[a]	Numbers IU Added Per Plate[b]		
	10^4	10^5	10^6
2×10^6	0	5	ND
1×10^7	0	3	ND
1.2×10^7	0	2	ND
3.6×10^7	0	2	24
5.3×10^7	●	66	>200
9×10^7	5	157	>200
9×10^7	11	181	>200
1.1×10^8	12	100	>200
1.5×10^8	0	2	19
2×10^8	●	26	>200
2.1×10^8	2	27	>200
2.5×10^8	2	23	>200
2.5×10^8	0	0	1
3×10^8	0	●	49
7×10^8	0	6	57
1×10^9	0	0	7

[a]Titer was determined by infecting BHK cells for 16 hours. and staining fixed cells with Xgal.

[b]Plaque forming units per 10^4, 10^5, or 10^6 I.U. of packaged SFV3-LacZ/helper 1.

ND, not determined

Depending on when during the packaging reaction the recombination event occurs, each resulting replication-competent virus could amplify itself in the same cell or adjacent cells by as much as a hundred fold, or more. Thus the observed frequency of recombinants is a maximum value: the actual rate of recombination is likely much lower. This mechanism also explains the wide variability observed in frequency of recombinants among the different virus stocks tested.

3. CLEAVAGE-DEFICIENT VARIANTS OF THE E2 SPIKE PROTEIN

The evidence for low levels of replication-competent virus in cells infected with SFV3/Helper1 virion suggested the value of additional measures to enhance the biosafety of this gene expression system. Liljeström and colleagues have shown that conditionally infectious particles (dependent on protease activation for infectivity) can be produced using a cleavage-deficient variant of SFV (Salminen, et al., 1992*). During transport of the p62-E1 spike heterodimer from the endoplasmic reticulum to the cell surface, p62 is cleaved to E2 and E3 in a post-Golgi compartment. Cleavage of p62 can be abolished by a single Arg for Leu substitution (mL mutation) in the last amino acid residue position of the E3 moiety of the p62 protein (Lobigs and Garoff, 1990; Salminen, et al., 1992*) (Figure 7). The mL mutation does not significantly affect virus maturation and budding, but the virus produced is virtually noninfectious, due to reduced binding and uptake into endosomes, as well as to reduced frequency of fusion between the viral and endosomal membranes. To infect cells with packaged virions, the virus stock is activated using chymotrypsin, which probably cleaves p62 at the leucine residue of the mutated cleavage site.

In separate, whole-animal studies (Glasgow et al., 1991*), the mL-SVF variant demonstrated a markedly reduced virulence for 40-day-old Balb/c mice when compared to the parent, unmutated cDNA clone (SFV4) or to the attenuated substrain of L10, from which the original cDNA clone was derived (Table 3). These results demonstrate that mutations which block p62 cleavage effectively reduce the virulence of SFV.

To reduce the potential for genetic reversion to wild-type infectivity, another mutant, SFV-SQL (Figure 7), was constructed in which all the arginine residues at the cleavage site were changed. Serine and glutamine substitutions were chosen, since such substitutions have been shown not to affect virus assembly (Jain et al., 1991). In addition, to reduce the possibility of reversion, two of the mutations were based on three-base substitutions and one on a two-base substitution.

Figure 7

Sequence of p62 Protein Variants

Genotype	E3	E2
Wild type	N G T R H R R	S V S Q H F N
mL	- - - - - - L	- - - - - - -
SQL	- - - S - Q L	- - - - - - -

Table 3[f]

VIRULENCE OF VIRUS STRAINS FOR 40-DAY-OLD MICE

Virus strain	Route of infection[a]	No. of mice infected	No. dead[b]	Mean time (days) of death (±SE)[c]
Progenitor Strain	i.n.	17	17	4 (±0)
	i.p.	31	17	7 (±0.5)
SFV4[d]	i.n.	12	12	4 (±0)
	i.p.	12	5	7.4 (±0.2)
SFVmL[d]	i.n.	17	4	8.3 (±1.0)
	i.p.	16	0	NA

[a]Virus (10^4 PFU) was inoculated by each route; in, intranasal; ip, intraperitoneal
[b]All surviving mice resisted challenge with a lethal dose (10^4 PFU) of the virulent L10 strain of SFV given i.p.
[c]SE, standard error.
[d]Virus produced from corresponding clone.
[e]NA, not applicable
[f]Table adapted from Glasgow et al., 1991

4. REVERSION OF MUTANT SQL-SFV

The frequency of reversion of the SQL-SFV system to infectious virus was measured, as follows: Eight independently produced SQL-virus stocks were activated with chymotrypsin and used to infect $2.5x10^6$ BHK cells on plates using 10^4, 10^5, or 10^6 particles. The cells were overlayed with agarose, and plaques scored after two days (Table 4). Based on the numbers of plaques observed in plates infected with $1x10^5$ infectious particles, the apparent reversion frequency is $1.2x10^{-4}$.

Table 4

Reversion Frequency of SFV-SQL Phenotype

Stock[b]	Number of Plaques Observed					
	10^{4a}		10^5		10^6	
	Act[c]	Unact[c]	Act	Unact	Act	Unact
4	1	0	14	0	>100	0
2	0	0	12	0	>100	0
3	0	0	8	0	>100	0
4	2	0	11	0	>100	0
6	0	0	8	0	>100	0
6	0	0	5	0	>100	0
7	2	0	19	0	>100	0
8	3	0	20	0	>100	0

[a]Amount of particles used for infection of 2.5'x 10^6 cells.
[b]Titre of all 8 stocks were 3 x 10^8/ml as measured by immunofluorescence of cells infected with activated stock.
[c]The virus was either activated (Act) by cleavage with chymotrypsin or left uncleaved (Unact).

Due to the multiple nucleotide substitutions of the SQL mutation, the observed reversion frequency of 10^{-4} is most likely due to a single-nucleotide, second-site mutation, rather than reversion of the SQL mutation itself.

5. PROPERTIES OF SFV/HELPER 2 VIRUS

An SFV helper virus containing the SQL mutation should provide a much higher level of biosafety than use of either SFV/Helper 1 or SFV-SQL alone. Thus Liljestrom and colleagues transferred the SQL mutation into the Helper 1 plasmid, creating the SFV/Helper 2 expression system.

The efficiency of the new helper was tested in packaging reactions using SFV3-lacZ as the recombinant RNA. Compared to the wild type SFV (SFV4), SFV3-lacZ/Helper 2 generated similar levels of viral protein 6.5 hours post-transfection and similar rates of assembly of pulse-lableled spike protein into mature virus particles (data not shown).

a. Leakiness of SFV/Helper 2 Virus

The residual infectivity of SFV-lacZ/Helper 2 particles, or "leakiness," was measured by exposing BHK cells to unactivated particles at various multiplicities of infection and scoring for LacZ-positive cells. Table 5 shows the result of testing four different stocks of virus. Only about one in $3x10^5$ particles is capable of entering BHK cells under these conditions.

(The mechanisms causing leakiness are unknown, but may result from distortion or cleavage of some of the spike proteins, not all of which need to be activated for infectivity (Salminen et al., 1992). Such mechanisms are distinct from genetic reversion, primary or secondary, which is inapparent without protease activation of the packaged stocks of virus and subsequent infection of cells. This is because the rare revertant RNA generated during a packaging reaction is surrounded by a vast excess of nonrevertant genomes, and hence will be packaged with nonrevertant coat proteins. Thus the new phenotype is manifest only on subsequent infection.)

Table 5

Leakiness of Helper-2 Packaged Virus Stocks[a]

Stock (titre[b])	MOI[c]	LacZ$^+$ cells/IU[d]
1 (1.2 x 10^9/ml)	95	1/1.0 x 10^6
2 (2.0 x 10^8/ml)	5	1/5.6 x 10^5
3 (2.5 x 10^8/ml)	6,5	1/2.3 x 10^5
3 (2.5 x 10^8/ml)	13	1/2.3 x 10^5
4 (0.7 x 10^8/ml)	3.6	1/4.0 x 10^5
4 (0.7 x 10^8/ml)	4.2	1/3.7 x 10^5

[a]Four different stocks were tested for infectivity without prior chymotrypsin treatment.
[b]Titre of stock after activation; determined by I.F.
[c]Refers to activated particles.
[d]Number of infected cells relative to titer of activated stock.

b. FREQUENCY OF REPLICATION-COMPETENT HELPER 2 VIRIONS

In Vitro Studies with BHK Cells

Critical to the biosafety of a recombinant SFV expression system is that few or no replication-competent virus be produced during the packaging of virions and subsequent infection of cells in culture. Based on the low frequency of recombinants formed between helper and vector RNAs and the low rate of reversion of the SQL phenotype, one would expect that the occurrence of both events in the same molecule would indeed be very rare.

An initial test of the SFV3-lacZ/Helper 2 system was performed using 10^6 BHK cells infected at a MOI of 1-10 with activated and unactivated virus particles. In four individual experiments, culture supernates were collected and titered for plaque-forming units (PFU). None was found in any of the samples (Table 1). In contrast, low levels of PFU were evident with the Helper 1 construct, as noted earlier. Sampling culture supernates permits highly sensitive detection of even very low numbers of replication-competent virus, because with every replication cycle, the number of virus present is amplified a thousand-fold or more.

In a second set of experiments searching for replication-competent virus (Table 6), approximately 10^8 BHK cells were infected with packaged SFV-lacZ/Helper 2 virus (±activation), at MOI of 3-4 and 60. After 16 hours of incubation, the cell culture medium was titered for PFU. Altogether, 1.34×10^{10} activated particles were examined. Despite the potential amplification of any replication-competent virus, no PFU were detected.

Table 6

Test for Replication-Competent Virus Using SFV-lacZ/Helper 2

Quantity of Virus (IU)[a]	MOI	Number of Cells Infected	PFU Observed[b]	
			-Act	+Act
6×10^8	3	2×10^8	0	0
1×10^8	4	2×10^8	0	0
1×10^8	60	1×10^8	0	0
1×10^8	60	1×10^8	0	0

[a]Titre determined after activatino, by IF.
[b]+Act, with protease activation; -act, without activation.

An important question is how few replication-competent virus were detectable with this assay and whether the high moi of 60 might have caused interference, thereby masking the detection of low levels of replication-competent virus.

The possibility of interference was first examined (Table 7) by scoring for lacZ-positive BHK cells infected with one or 1000 IU of packaged SFV3 lacZ/Helper 1 virus, alone or together with packaged SFV-hTR/Helper 2 virus (a recombinant clone of the human transferrin receptor), at MOI ranging from three to 30. No significant interference was detected, even at an MOI of 30.

Table 7

Double Infections with SFV Variants[a]

SFV-hTR/Helper 2 Virus Added (MOI)	LacZ Positive Cells	
	SFV-LacZ/Helper 1 Virus Added (IU)	
	1	1000
0	2	1000
3	1	1400
5	2	800
15	0	800
15	0	1000
20	1	100
30	1	600

[a]2.1 x 10^5 BHK cells were infected with activated SFV-hTR/Helper 2 virus at various MOI (titre determined by IF). In each experiment, lacZ-helper 1 virus was added at either 1 IU or 1000 IU, and cells were incubated overnight. Cells were then stained with Xgal for expression of LacZ.

In the next set of experiments (Table 8), 10^7-10^8 BHK cells were infected with SFV3-lacZ/Helper 2 virus, using a wider range of MOI. In parallel, wild type SFV (1, 3, or 5 IU) was added to assess interference. Altogether, ten independent recombinant Helper 2-derived stocks, totalling $2x10^{10}$ activated particles, were tested for the presence of replication-competent virus, by infecting a population of about 10^9 cells -- with no evidence of wild type virus.

The reconstitution experiments indicated that as few as one wild type SFV was detectable at an MOI of 17, and five wild type SFV at an MOI of 34, comprising a total of 3 x 10^9 SFV-lacZ/Helper 2 particles tested.

Thus within a range of MOI where interference is insignificant, no replication-competent virus were detected in 10^9 packaged virus, infecting greater than 5 x 10^8 BHK cells in culture.

Table 8

Occurrence of Plaque-forming Units (PFU) in Growth Media of Cells Infected with Recombinant SFV[a]

SFV4 (PFU)	lacZ Recombinant Virus Added MOI									
	0.01	0.1	0.5	5.0	7.5	10	17	34	60	75
0	0	0	0[b]	0[c]	0	0[d]	0	0	0[e]	0
1	0	+	0	+	nd	nd	+	0	nd	0
3	+	+	+	+	nd	nd	+	0	nd	nd
5	+	+	+	+	nd	+	nd	+	nd	0

[a]SFV3-lacZ recombinant SFV stocks packaged with Helper 2 were activated and used to infect 1 x 10^7 BHK cells (unless otherwise indicated) at various MOI in the presence or absence of wild type SFV (virus derived from the SFV4 clone).

[b]Repeated 2 times with independent stocks.

[c]Repeated 4 times with independent stocks of virus on 1 x 10^8 cells/experiment.

[d]Repeated 4 times.

[e]Done with 3 independent stocks using 1 x 10^8 cells/experiment.

nd, not determined

In Vitro Studies With Other Cell Lines

All of the infections of cultured cells described so far have utilized BHK cells. These cells are generally ideal for investigators seeking simply to produce high levels of proteins with the SFV/Helper 2 expression system. An important advantage of the SFV expression system, however, is that proteins can be produced in a wide variety of other cell types, as well. Thus we investigated whether replication-competent virus were produced during infection of three additional types of cells.

Using 1 x 10^8 packaged SFV3-lacZ/Helper 2 (activated), we infected 1 x 10^7 cells of the following types: H293 (human), COS (monkey), and CHO (hamster). Supernates were collected after 36 and 72 hours. Thirty percent of the supernates of each culture then was used (nonactivated) to infect BHK cells, and we assayed for plaques as evidence of replication-competent virus. None of the three cultures revealed any plaques.

In vivo Studies

Although the cDNA clone (SFV4) used to construct the present SFV expression system was derived from an attenuated variant of the prototype SFV strain L10, SFV4 virions are still relatively virulent when administered to neonatal mice. In vivo studies thus provide a highly sensitive indicator of the presence of replication-competent virus in a stock of packaged virus, as well as a test of the propensity of the Helper 2 constructs to revert the SQL mutation and/or to undergo recombination to form wild type virus in the complex cellular milieu of the whole animal.

Therefore, each of the SFV constructs discussed earlier was tested by intracerebral (i.c.) injection into neonatal BALB/c mice (Table 9). The SFV-SQL virus, when injected (2.5x10^7 IU) in either the nonactivated form or the activated form, killed all of the injected animals.

SFV-lacZ/Helper 1 virus (1x10^7 IU) also proved relatively virulent, rapidly killing four of the nine animals injected. But considering that 10^7 IU were innoculated, and that the frequency of recombinants (Table 2) is approximately 10^{-4}, it is surprising that less than half of the animals were killed. A reasonable explanation is that the recombination event disrupts the viral genome, resulting in virus that is attenuated, compared to wildtype SFV4.

In marked contrast to the above viruses, SFV-lacZ/Helper 2 virus injected i.c., activated or nonactivated, killed none of the 24 injected animals (1 x 10^7 IU per animal, 2.4x10^8 IU, total).

When the same set of viruses was injected intranasally (i.n.) into 40-day-old mice, at a ten-fold lower dosage of virus, none of the mice was killed. That all of the mice injected with SFV-SQL acquired resistance to challenge by a lethal dose of SFV strain L10 and that two-thirds demonstrated brain lesions, indicates that some level of virus replication occurred in these animals. But based on the measured reversion frequency (10^{-4}) of the SQL phenotype (Table 4), i.n. injection of 2.5 x 10^6 IU would be expected to result in revertants far exceeding in number the LD_{50} for SFV4, (10 IU). This apparent attenuation may reflect a lowered cleavage efficiency of the second-site revertants.

In contrast, neither SFV-lacZ/Helper 1 virus nor SFV-lacZ/Helper 2 virus (injected i.n.) demonstrated evidence of replication (2.5 x 10^6 IU per animal, 3.3 x10^7 IU total).

Reconstruction experiments were performed (Table 9, lower half) using SFV4 virus (injected i.n.) to assess whether the large excess of SFV-lacZ/Helper 2 could have masked the presence of low levels of replication-competent revertant virus. As few as ten infectious units of SFV4 were detectable in a population of $5x10^6$ activated SFV-lacZ/Helper 2 particles with this assay. Further, the LD_{50} for SFV4 injected i.n. into 40-day-old mice was shown to be only about 10 IU, demonstrating the high sensitivity of the mouse assay for replication-competent virus.

Also presented in Table 9 is a comparison of the virulence of the prototype SFV strain, L10, and the progenitor clone for the SFV expression system, SFV4. Whereas the LD_{50} for SFV4 is approximately 10^4 IU, the LD_{50} for L10 is clearly less than 10^4. In fact, the LD_{50} for SFV strain L10 by intraperitoneal injection of 40-day-old BALB/c mice is only 14 PFU (Barrett et al., 1980).

In summary, our inability to detect replication-competent SFV/Helper 2 virus either by *in vitro* infection of BHK cells, using a total of $2x10^{10}$ IU, or by injection of a total of $2.4x10^8$ IU into the brains of neonatal mice, indicates that coincident SQL reversion and helper-vector recombination of the SFV-lacZ/Helper 2 virus must be exceedingly rare, probably well below the frequency of 10^{-8} that one might predict from the individual apparent mutational rates.

Pathogenicity Profiles of SFV Variants

Total Virus Inoculated per Mouse[f]	Route of Infection[a]	No. of Mice Dead/Total	Mean Time (Days) of Death (±SE[b])	No. Surviving Challenge/Total[c]	No. showing CNS Lesions/Total[c]
SQL (2.5×10^7, unact.)	i.c.	10/10	6.8 (±0.5)	nd[d]	nd
SQL (2.5×10^7, act.)	i.c.	17/17	5.9 (±0.2)	nd	nd
lacZ/H 1 (1×10^7)	i.c.	4/9	2.5 (±0.3)	nd	nd
lacZ/H 2 (1×10^7 unact.)	i.c.	0/12	na[d]	nd	nd
lacZ/H 2 (1×10^7, act.)	i.c.	0/12	na	nd	nd
SQL (2.5×10^6, unact.)	i.n.	0/6	na	6/6	2/3
SQL (2.5×10^6, act.)	i.n.	0/7	na	7/7	2/3
lacZ/H 1 (1×10^6)	i.n.	0/5	na	0/5	0/3
lacZ/H 2 (1×10^6, unact.)	i.n.	0/5	na	0/5	0/3
lacZ/H 2 (1×10^6, act.)	i.n.	0/7	na	0/7	0/3
SFV4 (10^3) + lacZ/H 2 (5×10^6, act.)	i.n.	9/9	5.0 (±0.3)	nd	nd
SFV4 (10^2) + lacZ/H 2 (5×10^6, act.)	i.n.	9/9	5.0 (±0.4)	nd	nd
SFV4 (10^1) + lacZ/H 2 (5×10^6, act.)	i.n.	7/9	5.1 (±0.1)	nd	nd
SFV4 (10^3)	i.n.	9/9	4.8 (±0.3)	nd	nd
SFV4 (10^2)	i.n.	9/9	5.9 (±0.3)	nd	nd
SFV4 (10^1)	i.n.	5/9	5.0 (±0.0)	nd	nd
lacZ/H2 2 (5×10^6, act.)	i.n.	0/9	na	nd	nd
SFV4 (10^4)	i.p.	10/19	7.5 (±0.2)	nd	nd
L10 (10^4)	i.p.	6/6	5.2 (±0.3)	nd	nd

[a]The intracerebral (i.c.) injections were given to neonatal mice, whereas the intranasal (i.n.) and intraperitoneal (i.p.) injections were given to 40-day-old mice.

[b]SE, standard error.

[c]Mice were challenged at 14 days p.i. with 10^4 PFU given intraperitoneally (i.p.) of the virulent SFV L10 strain.

[d]nd, not done; na, not applicable.

[e]Lesions were scored at 14 days post-injection. Two of three mice infected with activated or unactivated SQL virus showed focal areas of gliosis and lymphoid perivascular cuffing in the grey and white matter areas of the brain. Shrunken neurons with fragmented nuclei and markedly eosinophilic cytoplasm were occasionally observed in the lesions.

[f]SQL, virus derived from SFV-SQL cDNA; lacZ/H1, SFV3-lacZ recombinant virus packaged with Helper 1; lacZ/H2, SFV3-lacZ recombinant virus packaged with Helper 2; SFV4, virus derived from SFV cDNA clone. L10, virulent prototype strain of SFV. Amounts of activated or unactivated virus used are indicated in parentheses.

B. PATHOGENICITY OF SFV

1. Naturally Occurring SFV Infections

First isolated and identified in 1942 (Smithburn and Haddow, 1944), SFV has been isolated in the wild from sentinel mice, wild birds, and several species of mosquitoes in sites widely distributed throughout Africa (Berge,1975). In addition, at least one isolate of SFV has been reported from Surinam, South America (Bradish,1971). Antibodies against SFV have been detected commonly in chimpanzees, wild rodents, and domestic animals in South Africa.

Serological surveys indicate that SFV infection is common in human populations (5%-17%) of several regions within central and southern Africa (Berge, 1975; McIntosh et al., 1961). Neutralizing antibodies for SFV also have been detected in the peoples of India, Thailand, Vietnam, Malaya, Borneo, and the Philippines (Berge,1975). Nevertheless, for more than forty years, human SFV infection in nature was considered to be mild and largely asymptomatic.

The first, and only, report of naturally occurring, symptomatic SFV infection in man appeared in 1990 (Mathiot et al, 1990). During an outbreak of febrile illness in the Central African Republic, sera were drawn from 163 individuals presenting with acute, viral-like illness. From 23 of these patients' sera, SFV was isolated. Most of the SFV-positive sera isolated were from Europeans, particularly soldiers recently arrived from France; only three positive samples were obtained from Africans.

Virus identification by cross-neutralization assays indicated that the isolated strains were all identical, and related to known strains of SFV, including the prototype virus (Smithburn and Haddow, 1944).

The symptoms in all cases included fever, headache, arthritis, and myalgia, and were "...generally mild and undifferentiable during the acute phase from other febrile illnesses, such as malaria and influenza (Mathiot et al,1990)." However, headaches in some cases, lasted up to 15 days. The acute symptomatic phase, of 2-4 days, was followed by a prolonged period of convalescence. No fatalities were recorded.

Whether this outbreak of illness in the Central African Republic was due to an unusually virulent strain of SFV is unknown. But this study established for the first time that naturally occurring SFV occasionally can cause symptomatic infection in man. No other examples of naturally occurring, overt SFV infection in humans have been reported.

Noteworthy are several outbreaks of human illness, in Africa, Russia, Finland, and Sweden, ascribed to another alphavirus closely related to SFV, Sindbis virus (Reviewed in: Griffin, 1986; and Acha and Szyfres, 1980). Manifestations of Sindbis virus infection can include fever, rash, joint pain, tendonitis, headache, nausea, vomiting, sore throat, periocular pain, and mild jaundice. Seropositivity is common in endemic regions, but overt disease is relatively unusual. Nevertheless, joint pain associated with Sindbis infection can persist for several weeks, and one case experienced an hemorrhagic rash with multiple recurrences over a period of four months (Guard et al.,1982). Sindbis virus is classified for use at biosafety Level 2 contaminent (BMLB, 1988).

2. Experimental SFV Infection

The virulence of SFV in laboratory animals varies markedly with the strain of SFV employed, the age of the animal, and the route of administration. From four early isolates of SFV, numerous strains are available for study (Bradish, 1971). Mice up to 15 days of age are uniformally killed by low numbers of all strains of SFV (1-3 PFU/LD_{50}) administered by any route. After 15-20 days of age, mice become totally resistant ($\geq 10^7$ PFU/LD_{50}) to certain strains (A7, and derivatives), but are lethally infected (100 PFU/LD_{50} i.p.; 1-3 pfu/LD_{50} i.c.) by all other strains, including L10 (Bradish,1971). Cause of death is acute encephalitis with glial and neuronal necrosis, associated with high titres of virus in the brain (reviewed by Griffin, 1986).

3. **Human Infections In The Laboratory and SALS Classification of SFV**

On December 7, 1977, William Scherer, Chairman of the Subcommitee on Arbovirus Laboratory Safety (SALS) of the American Committee on Arthropod-borne Viruses, wrote to several hundred laboratories worldwide summarizing the results (described below) of a SALS survey conducted in 1976 of safety practices and experience with arboviruses. Scherer included a proposed classification by SALS of biocontainment levels for each of the arboviruses listed, anticipating publication of a final set of recommendations in 1979. Among the arboviruses listed, SFV was recommended for biocontainment level 2, as was Sindbis virus. When SALS issued its final reccommendations, approximately one year later, however, SFV was listed at Biosafety Level 3.

The deciding factor that changed the classification of SFV was a fatal SFV infection in a laboratory worker on June 16, 1978, in Tubingen, Germany (Willems et al., 1979). While conducting experiments with nasal innoculations of mice with SFV, this worker contracted a rapidly progressive encepalitis, that proved fatal approximately one week later. Virus isolated from the cerebrospinal

fluid and brain tissue on autopsy revealed close similarity to the Osterrieth strain of SFV that was used in the experiments, both by size analysis of viral proteins and by virus neutralization and hemagglutination assays.

Viewed against the three decades during which SFV had been safely employed in many laboratories worldwide, often with minimal containment, this exceptional incident raised several questions. Most important were whether the health of the worker was compomised in a way that predisposed her to this infection, whether she had received an unusually heavy innoculum, and whether the strain of SFV employed was unusually virulent.

Regarding the innoculum, no useful information is available. Even whether nasal injection or aerosol was used to administer the virus has never been certain.

Relatively little is known of the patient's health or medications. In their published report of this incident, Willems et al. (1979) describe that she had endured a purulent bronchitis of one year's duration at the time of the fatal infection. Further information regarding her state of health and immunocompetence is unavailable.

Regarding the virulence of the SFV strain, some helpful information is available. According to the published report of this incident (Willems et al., 1978), the Osterrieth strain of SFV, prior to use in the fatal experiment, had been passaged extensively in animals: Following 13 passages in primary CEF cells, the virus was passaged 7 times intracerebrally in four- to five-week-old mice, and once subcutaneously in six- to seven-week-old mice. Brain material from these mice was then passaged four times intracerebrally in six- to seven-day-old mice, then once in BHK cells. The patient worked with the supernatant of this passage. Multiple passage of SFV in mouse brains can be an effective means to select for SFV strains with increased virulence for mice (Smithburn and Haddow, 1944).

Approximately one week after the worker died, two scientists investigating this incident (W.R.Willems and G.Kaluza, Institute for Virology, Giessen) compared the virulence of four different strains of SFV employed in the Tübingen laboratory, plus the virus isolated from clinical material obtained from the patient. Each strain was administered by subcutaneous injection ($1x10^7$ pfu) into four mice, and the time till death was measured. A brief report of this unpublished study is summarized here:

Virus	Hours Till Death	Mean
Patient Isolate(1397/78)	64,73,90,116	86 hrs.
Osterrieth	95,116,139,163	128 hrs.
Tb virulent	116,116,186,211	157 hrs.
Hd	211,-,-,-	one death only
Tb avirulent	-,-,-,-	no deaths

Thus the virus isolated from the patient (1397/78), when compared to the relatively virulent starting strain (Osterrieth) of SFV, appears to have acquired considerable virulence for mice (mean hours to death decreased from 128 to 86), probably reflecting its multiple passages in animals.

Also noteworthy, the relatively avirulent strain Hd used in these experiments is identical to the avirulent substrain of L10 that Liljeström and Garoff utilized to isolate SFV4, the progenitor of the SFV/Helper 2 expression system (A. Helenius, Pers. Commun.)

Because only four mice were injected with each SFV strain, however, the evidence suggesting increased virulence of the infecting SFV strain is preliminary. And although SFV strains with increased virulence in mice frequently have demonstrated increased virulence in guinea pigs and rabbits (Bradish et al., 1971; Smithburn and Haddow, 1944), published studies comparing SFV virulence in mice and humans do not exist. Thus an increased SFV virulence in mice suggests, but does not prove, increased virulence for humans.

That the SFV strain involved in the fatal incident had been deliberately passaged to enhance its virulence apparently was known to SALS prior to circulating its final recommendations in January 1979, three months before the Willems et al. report was published. But the unpublished results from Willems and Kaluza, on the virulence in mice of the clinical isolate of SFV, evidently were unknown to SALS prior to the formulation of its recommendations. Phone interviews conducted (by G.F.T.) with two former SALS members involved with the reclassification of SFV indicated that SALS was unaware of these results.

These results might well have altered the committee's classification of SFV. For the SALS report that issued soon thereafter (SALS, 1980) stated that in developing recommended levels of practice and containment the committee members had "...recognized that different strains of the 'same' virus can vary markedly in pathogenicity. Differences in virulence occur naturally among strains and can also result from laboratory manipulations....Assignment of a virus to a level was generally made on the basis of characteristics of the viral prototype or

predominant strain. It is recognized that another strain of the same virus with an altered biohazard potential may justifiably be assigned to a different level." (SALS, 1980*)

Had SALS been aware of results suggesting that the SFV strain involved in the Tübingen incident was one of increased virulence, it might well have chosen to classify the prototype strain of SFV as a Level 2 agent, while placing SFV strains of enhanced virulence, such as the multiply passaged Tübingen strain, at Level 3.

The correspondence of the SALS group and interviews with former SALS members both indicate that the Tübingen fatality was the principal concern of the committee regarding the proper classification of SFV. SALS recognized this case as "...a unique incident in a long history of work with SFV under minimal biocontainment conditions."(SALS, 1980*) But in the absence of information clarifying the circumstances of this anomaly, the committee felt compelled to classify SFV at BL3.

The 1980 SALS recommendations on the containment and practices for arboviruses were subsequently adopted, largely intact, into the CDC/NIH biosafety regulations in effect today. But the ambivalence of the Subcommittee in 1979 still echoes in its recommendations of 1993: "SALS continues to classify SFV as a BL3 virus, with the caveat that most activities with this virus can be safely conducted at Biosafety Level 2."(BMBL, 1993)

4. The SALS Survey on Laboratory Experience with SFV

SALS conducted, between 1976 and 1978, two worldwide surveys of laboratory experience and practices with approximately 400 arboviruses (arthropod-borne viruses). As described in the report summarizing the results of these surveys (SALS, 1980*):

> A checklist questionnaire was developed by SALS to evaluate existing and past levels of biological containment in arbovirus research facilities, the frequency with which catalogued viruses have been handled at each laboratory, the numbers of people at risk, the numbers of overt and subclinical infections, and the probable routes of infection.

The first survey was mailed, in 1976, to 494 laboratories worldwide. 158 laboratories responded, 48 of which employed SFV. A second survey, in 1978, was mailed to 585 laboratories to seek additional information. No response was requested unless new information on virus use was available. Ninety-four laboratories responded, of which 16 had used SFV.

Data from the surveys were collated for each virus according to number of persons at risk, number of times the virus was handled in the laboratory, type of laboratory facility as described in the survey instructions, and numbers of overt and subclinical infections with the virus. Neither survey distinguished between animal and tissue culture exposures.

In the 1976 survey, respondents were requested to classify their facilities into one of five categories, A through E. In the second survey, the containment and practices were classified into four categories, Level 1 through Level 4. The definitions of these different categories, as included with each survey, are provided in Appendix D.

Based on the information provided in the survey, SALS calculated an "experience factor" for each virus for each laboratory, by multiplying the number of times the virus was handled, (once (1), two to twenty times (2), or greater than twenty times (20)) by the number of people at risk (one to five (1), six to twenty (6), twenty-one to one hundred (21), or greater than one hundred (100)). (Numbers in parentheses are the multipliers used). In the following, we have adhered to the same conventions, with the exception that multipliers used for the number of people at risk are the actual numbers of workers at risk, as reported by each laboratory. The product of the actual number of people at risk times the number of times the virus was handled, is here termed "exposure experience."

Table 10 displays the results obtained in the 1976 survey from the 48 laboratories reporting experience with SFV. A total of 330 workers were exposed, with an average exposure number per worker of 18. Over one-half (53%) of the exposure experience with SFV in these laboratories was at containment levels equivalent to the present category of Biosafety Level 1 (Level A+Level B). No overt infections were reported among this group; one possible inapparent infection was described, this individual had a hemmaglutination-inhibition (HI) assay result positive at a titre of 1:10, which is borderline positive.

Among the respondents to the 1978 SALS survey (Table 11), sixteen laboratories employed SFV in their work, and one hundred forty additional workers were reported as at risk for SFV infection. Of the total exposure experience for this group, 15% was at Level 1 and 83% at Level 2, levels which are nearly equivalent, respectively, to present-day Biosafety Level 1 and Biosafety Level 2.

One overt infection was reported, the fatal encephalitis discussed above. Two individuals in the same Tübingen laboratory were tested for antibodies to SFV: one was positive without an overt infection.

The two SALS surveys provide a valuable record of experience with wild type SFV in more than fifty laboratories, comprising 470 laboratory workers, who handled SFV an average of 17 times, often at containment levels equivalent to the current Biosafety Level 1. In this large and diverse base of experience, involving various strains of SFV, with the exception of the remarkable and unique Tübingen incident, no overt infections were recorded.

Table 10

Results of 1976 SALS Survey
for SFV

Facility Level	No. Labs	No. Workers Exposed	Total Exposure Experience[a] (%)	Avg. Expos. per Worker[b]	Overt Lab Infection	Inapparent Infection
A	9	73	1393 (23)	19.1	0	0
B	18	103	1802 (30)	17.5	0	1[c]
C	9	141	2545 (42)	18.0	0	0
D	2	13	273 (5)	21.0	0	0
E	0	0	0			
TOTAL	48	330	6013	18.2		

[a]Exposure experience = (number of laboratory workers exposed to SFV) X (minimum number of exposures calculated separately for each laboratory.

[b]Average exposure per worker = (Total exposure experience) ÷ (number of workers exposed). Exposure experience was rated as 1 (if a worker handled SFV once), as 2 (for two to twenty times) or as 21 (for greater than twenty times).

[c]Hemagglutination inhibition for SFV was positive at 1:10; negative at 1:20.

Table 11

Results of 1979 SALS Survey
for SFV

Facility Level	No. Labs	No. Workers Exposed	Total Exposure Experience[a] (%)	Avg. Expos. per Worker[b]	Overt Lab Infection	Inapparent Infection
1	6	28	303 (15)	10.8	0	0
2	9	87	1675 (83)	19.3	1[c]	0[d]
3	1	25	50 (2)	2.0	0	0
4	0	0	0			
TOTAL	16	140	2028	14.5		

[a]Exposure experience = (number of laboratory workers exposed to SFV) X (minimum number of exposures) calculated separately for each laboratory).

[b]Average exposure per worker = (Total exposure experience) ÷ (number of workers exposed). Exposure experience was rated as 1 (if a worker handled SFV once), as 2 (for two to twenty times) or as 21 (for greater than twenty times).

[c]Patient died of SFV encephalitis.

[d]1 of 2 persons tested was seropositive, in same laboratory as c.

The SALS data do not provide a complete record of the serological status of SFV laboratory workers, however, as sera generally were not sampled without signs of an overt infection or accident. To assess the frequency of inapparent SFV infections, serological surveys of laboratory workers exposed to SFV are needed.

5. Serological Surveys of Individual Laboratories

Yale Arbovirus Research Unit

Professor Robert E. Shope provided the following serological survey of individuals who worked with various arboviruses, including SFV, at the Rockefeller Foundation Virus Laboratories or the Yale Arbovirus Research Unit.

Fifty-two sera initially were screened for SFV by hemagglutination inhibition. The eight positive individuals then were tested for reactivity with antigens of Mayaro, chikungunya, and getah viruses, all members of the genus *Alphavirus* and closely related to SFV. Presumptive diagnoses are indicated (Table 12).

Table 12

Semliki Forest, Mayaro, Chikungunya, and Getah Hemagglutination-inhibition Test Results - December 21, 1992 - Yale Arbovirus Research Unit

Worker	Worked with SFV	Period of Exposure (Years)	Sample Date	Semliki Forest	Mayaro	Chikung.	Getah	Inter-pretation
CMA	+	13	12/5/78	0				
SMB	+	20	12/5/78	0				
JC	+	29	12/5/78	0				
RC	+	18	1/13/64	40	0	320	20	Ch
DC	+	19	5/10/68	0				
KM	+	18	7/20/79	0				
CM	+	18	10/19/72	0				
FR	+	5	8/20/70	0				
PR	+	6	11/8/65	0				
GR	+	10	4/23/75	0				
RS	+	18	7/14/75	10	0	0	0	SFV/NS
RES	+●	34	12/3/92	40	80	80	40	Ma●
MT	+	4	7/13/53	0				
JW	+	20	12/5/78	0				

Worker	Worked with SFV	Period of Exposure (Years)	Sample Date	Semliki Forest	Mayaro	Chikung.	Getah	Inter-pretation
LW	+	16	1/23/65	40	80	80	40	Ma
MC-W	*	12	12/3/92	160	0	0	<20	SFV
THGA	-	22	12/3/92	0				
CA	-	4	3/14/72	0				
PA	-	2	6/8/67	0				
PB	-	4	3/14/72	0				
FLB	+	3	9/29/75	0				
SB	-	3	12/5/78	0				
IC	-	4	10/19/72	0				
KC	*	10	12/5/78	0				
JC	-	4	1/10/80	0				
WD	-	12	12/5/78	0				
CLF	-	4	7/9/78	0				
MG	-	12	12/3/92	0				
EG	-	13	12/5/78	0				
KG	-	10	12/5/78	0				
MG	-	8	6/26/72	0				
J-PG	*	6	12/3/92	0				
SBH	-	2	12/6/65	320	40	5120	80	Ch
NK	-	3	5/11/64	0				
RK	-	4	7/13/79	0				
JL	-	4	3/31/65	0				
AJM	-	10	12/5/78	0				
JMM	-	3	10/24/79	0				
NM	-	3	12/12/60	0				
VP	-	8	2/15/72	0				
MR	*	5	4/23/75	0				
DR	-	3	5/11/64	0				
KR	-	5	4/23/75	0				
ALS	-	5	12/5/78	0				

Worker	Worked with SFV	Period of Exposure (Years)	Sample Date	Semliki Forest	Mayaro	Chikung.	Getah	Inter-pretation
TS	-	4	8/10/65	0				
RBT	-	12	12/3/92	10	0	80	10	Ch
GHT	-	28	12/3/92	0				
SJT	-	7	12/3/92	0				
OT	-	2	1/3/76	0				
RW	-	11	2/6/76	0				
DW	-	8	12/8/78	0				
OLW	-	5	1/26/78	10	0	0	0	SFV/NS

+ Worked with SFV on open bench between 1949-1970 all experiments used animals; none used tissue cultu
* Extensive exposure to SFV prior to joining lab; did not work with SFV in RES lab.
• Known to be infected in 1959 with Mayaro Virus.
SFV/NS: HI results indeterminate. Possibly due to weak SFV-specific antibody response or nonspecific crossreaction.

According to Prof. Shope, the persons indicated with a + all "...worked with SFV on a regular basis between 1949 and 1970, usually on an open bench, and mostly with mouth pippetting. Many of these persons continued to work with SFV after 1970, but under BL 2 and BL 3 conditions. The strain of SFV employed was a low-passage descendant of the earliest prototype virus, isolated by Smithburn and Haddow, in 1942.

Other persons tested worked in the Rockefeller Foundation Virus Laboratories or its successor organization, the Yale Arbovirus Research Unit, most of them for years, in laboratories where Semliki Forest virus research was being conducted. They were not, however, personally engaged in this research at the bench level, or did not work for very long periods with Semliki Forest virus."

Prof. Shope noted that all persons exposed to SFV performed experiments with animals, as tissue culture studies in the Arbovirus Research Unit did not begin until after 1970.

Only one individual, MC-W, was strongly reactive to SFV; but she had not worked with SFV in the Yale Arbovirus Research Unit since her employment at Yale two years before. Prof. Shope noted that "MC-W worked with Semliki Forest virus for several years in Abidjan and Bangui, then later as the lead technical person in the diagnostic unit of the Institut Pasteur, Dakar. She was potentially exposed to natural infection in Africa. She has no history of encephalitis or other severe illness that might be linked to Semliki Forest virus infection."

Seven other individuals were reactive for SFV. Two (RS and OLW) were borderline positive. The other five demonstrate significant reactivity to SFV, but because they are more reactive with another member of the Semliki complex of viruses, these workers were judged to be infected by the virus giving the highest dilution titre. Based on these results, however, one cannot conclusively exclude a coincident infection with SFV.

Institute of Biotechnology, University of Helsinki

A second survey of laboratory workers exposed to SFV was provided by Professor L.Kääriäinen (courtesy of Prof. A. Helenius, Yale University). According to Prof. Kääriänen, each individual tested had worked with SFV, on average, for about five years, under BL2 conditions. This laboratory has studied SFV since 1965, handling altogether "at least several grams of virus" with no sign of overt infection. All studies with SFV involved tissue culture; none was

performed with animals. The strain of SFV used in this laboratory was the attenuated L10 derrivative, also the progenitor of the SFV/Helper expression system.

Serologic testing revealed (Table 13) that eight of sixteen individuals were weakly seropositive at dilutions of 1:5 to 1:10 by the hemagglutination inhibition assay (HI), or by the more sensitive hemolysis-in-gel assay (HIG) (Väänänen, 1982).

Table 13

Serologic Monitoring of Individuals Exposed to SFV in the Laboratory of L. Kääriäinen Institute of Biotechnology, University of Helsinki Helsinki, Finland

Worker	Date Tested	HIG[1]	HI[2]	Interpretation[3]
SK	10/78 8/81	32 64	5 ND	+
LK	10/78 8/81	128 128	10 ND	+
HS	10/78 8/81	16 16	<5 ND	- -
PV	11/78 6/81	8 32	<5 5	- +
MR	10/76 6/81	- -	<5 <5	- -
RR	10/78	64	5	+
JU	10/78	64	10	+
RP	10/78	91	10	+
RL	6/81	-	<5	-
MPer	6/81	-	<5	-
TR	6/81	-	<5	-
MPel	6/81	-	<5	-
KK	6/81	-	<5	-
LR	6/81	91	10	+
JS	6/81	-	<5	-
AH	8/81	23	ND	+
Anti-SFV Rabbit		23,171	320	
Neg. Rabbit		-	<5	

[1]Hemolysis in gel assay titre, highest reactive titre (Väänänen, 1982).

[2]Hemagglutination inhibition assay titre, highest reactive titre.

[3]+ weakly positive
- negative

One of these individuals (PV) seroconverted between 1978 and 1981. These results are presumptive evidence of low-grade SFV infection; however, because this laboratory also handled other alphaviruses, it is difficult to exclude the possibility that some of the apparent positives result from infection by viruses antigenically similar to SFV.

In summary, serological data from two sites, comprising thirty-two laboratory workers exposed to SFV over a period of five years or more, suggest that low-level, inapparent SFV infection occasionally can occur. The experience of these two laboratories contrasts in several regards: Each used a different strain of SFV; one worked exclusively with animals, the other only with tissue culture; and they recorded markedly different frequencies of SFV seroconversion. In one matter, however, their experience coincided: neither recorded an overt SFV infection among any of their laboratory workers.

C. METHODS TO INACTIVATE SFV

Studies on the inactivation of SFV were performed to determine which reagents will effectively inactivate the virus, given a BL2 laboratory setting. The reagents evaluated were BACDOWN detergent disinfectant, BACDOWN antimicrobial handsoap, chlorine bleach, NP-40, Triton X-100, and sodium dodecyl sulfate (SDS) solutions. UV light inactivation was also studied.

Procedures:

Viral stock solution of SFV3 LacZ/Helper 2 were prepared by electroporation in BHK-21 as described in section IIIA.

Activation and infection were performed using standard procedures described in section IIIA.

Reagents:

Reagent	Stock Solutions
BACDOWN™	15ml/L
BACDOWN™ Handsoap	10ml/L
Chlorine bleach	100ml/L
NP-40	1% Solution
Triton X-100	1% Solution
SDS	1% Solution

Controls:

Plating efficiency of activated SFV-3 LacZ/Helper 2 virus was determined for each of the reagents above to determine the concentration that did not interfere with viral infection of cells. The following concentrations of reagents had no effect on virus infectivity:

Reagent	Reagent Conc.
BACDOWN Detergent	0.45ml/L
BACDOWN Handsoap	0.1ml/L
Chlorine bleach	0.17ml/L
NP-40	.033%
Triton X-100	.033%
SDS	.033%

Therefore, these concentrations were used in the following experiments as the maximal final concentration of inactivating reagent to which the cells were exposed. This assured that apparent reductions in infectious units of SFV resulted from virus inactivation, rather than interference with virus infection or toxicity for BHK-21 cells.

Inactivation protocol:

$1x10^6$ infectious units of activated SFV3-lacZ/Helper 2 particles (50 μl) were combined with an equal volume of disinfectant reagent stock, or dilution of that reagent, for 5 minutes at room temperature. 20 μl of the reagent/virus solution were then diluted into 6 mls PBS (Ca^{++}, Mg^{++}) and 1 ml was added to each well of a 6-well plate. After a one-hour incubation at 37°C, the plate was washed once with PBS, and 3 ml of complete medium were added to each well. At 24 hr post-infection, the cells were stained *in situ* for β-gal activity, using X-gal as a substrate (see protocol in Section III A).

In each experiment, two controls were incorporated. In one, $2x10^5$ infectious units of SFV3-lacZ Helper 2 particles were incubated for 5 minutes in PBS, and diluted prior to plating. In the other control, $2x10^5$ infectious units were also incubated for 5 minutes in PBS, and then diluted into a concentration of inactivating reagent known not to interfere with virus infection of cells (see above) prior to plating.

Results:

BACDOWN DETERGENT DISINFECTANT

Inactivation Conc.	Final conc.on cells	No. of β-gal positive cells/6 wells
7.5 ml/L	.025ml/L	0
2.5 ml/L	.0083ml/L	7
0.75ml/L	.0025ml/L	72
PBS control		$\sim 2x10^5$
PBS control	.025ml/L	$\sim 2x10^5$

Conclusion: 7.5 ml/L, or higher, of BACDOWN detergent completely inactivates $2x10^5$ infectious units of SFV.

NP-40

Inactivation Conc.	Final conc.on cells	No. of β-gal positive cells/6wells
0.5% NP-40	.0017%	0
0.17% NP-40	.00055%	0
0.05% NP-40	.00017%	6
PBS control		$2X10^5$
PBS control	.0017%	$2X10^5$

Conclusion: 0.17% NP-40, or higher, completely inactivates $2x10^5$ infectious units of SFV.

TRITON X-100

Inactivation Conc.	Final conc.on cells	No. of β-gal positive cells/6wells
0.5% Triton X-100	.0017%	7
0.17% Triton X-100	.00055%	300
0.05% Triton X-100	.00017%	1000
PBS control		$\sim 2X10^5$
PBS control	.0017%	$\sim 2X10^5$

Conclusion: 0.5% Triton X-100 reduces SFV infectious units by ~30,000 fold.

SDS

Inactivation Conc.	Final conc. on cells	No. of β-gal positive cells/6wells
0.5% SDS	.0017%	0
0.17% SDS	.00055%	0
0.05% SDS	.00017%	100
PBS control		$2X10^5$
PBS control	.0017%	$2X10^5$

Conclusion: 0.17% SDS, or higher, completely inactivates $2x10^5$ infectious units of SFV.

BACDOWN ANTIMICROBIAL HANDSOAP

Inactivation Conc.	Final conc.on cells	No. of β-gal positive cells/6wells
5 ml/L	.017ml/L	0
1.7 ml/L	.0055ml/L	0
0.5 ml/L	.0017ml/L	0
PBS control		$2X10^5$
PBS control	.017ml/L	$1X10^3$

Conclusion: 0.5 ml/L, or higher, of BACDOWN Handsoap completely inactivates $2x10^5$ infectious units of SFV.

CHLORINE (HOUSEHOLD) BLEACH

Inactivation Conc.	Final conc.on cells	No. of β-gal positive cells/6wells
50 ml/L	.017ml/L	0
17 ml/L	.0055ml/L	0
5 ml/L	.0017ml/L	7
PBS control		$2X10^5$
PBS control	.017ml/L	$1X10^3$

Conclusion: 17 ml/L, or higher, of chlorine bleach completely inactivates $2x10^5$ infectious units of SFV.

UV LIGHT

The killing of SFV by UV light was evaluated using 100μl aliquots ($1x10^6$ infectious units) of viral stock ($\sim 2x10^7$ infectious units/ml) dispensed into 35 mm dishes. With lids removed, virus aliquots were exposed to a UV light source (30W Germicidal light, Sylvania), for 0, 5, 10, 20, 30, 40, 50 or 60 minutes. Treated virus samples (100μl) were then removed and plated on BHK 21 cells, following the standard protocol for infection (Section IIIA). Cells were incubated 18 hrs at 37°C then stained for β-gal expression as described in section IIIA).

Results:

Time of UV exposure (min)	No. of β-gal pos. cells
0	$>5x10^5$
5	1
10	0
20	0
30	0
40	0
50	0
60	0

Conclusion: UV light exposure for 10 minutes or longer completely inactivates $5x10^5$ infectious units of SFV.

In summary, SFV on surfaces can be completely inactivated by a 10-minute, or longer, exposure to UV light; SFV in solution is inactivated by incubating for 5 minutes at room temperature with any of the following reagents at, or above, the indicate concentrations.

Reagent	Minimum Effective Concentration
BACDOWN Detergent	7.5 ml/L
NP40	0.17%
SDS	0.17%
Chlorine bleach (5%)	17 ml/L
BACDOWN Handsoap	0.5 ml/L

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National Institutes of Health

Recombinant DNA Research: Action Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Action Under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth an action to be taken by the Director, National Institutes of Health (NIH), under the May 7, 1986, NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Technology Transfer, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: Today, one action is being promulgated under the NIH Guidelines for Research Involving Recombinant DNA Molecules. This proposed action was published for comment in the **Federal Register** of November 2, 1992 (57 FR 48584), and reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its meeting on December 3–4, 1992.

I. Background Information and Decisions on Action Under the NIH Guidelines

A. Addition of Appendix D–XLVI to the NIH Guidelines

In a letter dated October 8, 1992, Dr. James M. Wilson, University of Michigan Medical Center, Ann Arbor, Michigan, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trail. This request was published for comment in the **Federal Register** of November 2, 1992 (57 FR 49584).

The protocol was reviewed and recommended for approval during the RAC meeting of December 3–4, 1992, by a vote of 16 in favor, 0 opposed, and no abstentions. Approval of the protocol was contingent on the following stipulations: (1) The patient eligibility criterion in the Informed Consent document requiring that patients are documented to be sterile will be replaced with a statement suggesting that all patients should exercise appropriate birth control methods; (2) the expected benefits of the procedure should be clearly stated in the first section of the document; (3) submission of a revised Informed Consent document incorporating minor changes suggested by committee members; and (4) the demonstration that there is less than one replication-competent adenovirus particle per 20 milliliters of vector supernatant.

On June 24, 1993, Dr. Wilson submitted sequencing information on the recombinant adenovirus vector, Ad.CF–CFTR, that will be used in the protocol. On August 2, 13, and 16, Dr. Wilson submitted materials to satisfy the rest of the stipulations. These materials were reviewed by primary reviewers of the protocol, and it was determined that the stipulations of the RAC were met. The following section may be added to Appendix D:

"Appendix D–XLVI

"Dr. James M. Wilson of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct experiments on 20 adult patients with advanced cystic fibrosis lung disease. An isolated segment of the patients' lung will be transduced with the E1 deleted, replication-incompetent adenovirus vector, Ad.CB–CFTR using a bronchoscope for gene delivery. Ad.CB–CFTR contains the human gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Pulmonary biopsies will be obtained by bronchoscopy at 4 days, 6 weeks, and 3 months following treatment. Patients will be monitored for evidence of CFTR gene transfer and expression, immunological responses to CFTR or adenovirus proteins, and toxicity."

I accept this recommendation, and Appendix D–XLVI of the NIH Guidelines will be added accordingly.

II. Summary of Actions

A. Addition of Appendix D–XLVI to the NIH Guidelines

The following section is added to Appendix D:

"Dr. James M. Wilson of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, may conduct experiments on 20 adult patients with advanced cystic fibrosis lung disease. An isolated segment of the

patients' lung will be transduced with the E1 deleted, replication-incompetent adenovirus vector, Ad.CB–CFTR using a bronchoscope for gene delivery. Ad.CB–CFTR contains the human gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Pulmonary biopsies will be obtained by bronchosopy at 4 days, 6 weeks, and 3 months following treatment. Patients will be monitored for evidence of CFTR gene transfer and expression, immunological responses to CFTR or adenovirus proteins, and toxicity."

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: August 25, 1993.

Ruth L. Kirschstein,

Acting Director, National Institutes of Health.

[FR Doc. 93–22260 Filed 9–10–93; 8:45 am]

BILLING CODE 4140–01–M

Recombinant DNA Research: Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth twelve actions to be taken by the Director, National Institutes of Health (NIH), under the May 7, 1986, NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Technology Transfer, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838).

SUPPLEMENTARY INFORMATION: Today twelve actions are being promulgated under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These twelve proposed actions were published for comment in the Federal Register of August 19, 1992 (57 FR 37680), and May 4, 1993 (58 FR 26676), and reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its meetings on September 14–15, 1992 and June 7–8, 1993.

I. Background Information and Decisions on Actions Under the NIH Guidelines.

A. Addition of Appendix D–XLVII to the NIH Guidelines

In a letter dated December 16, 1992, Dr. Hilliard F. Seigler of the Duke University Medical Center, Durham, North Carolina, indicated the intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients with Disseminated Malignant Melanoma. This request was published for comment in the Federal Register of February 12, 1993 (58 FR 8500).

The protocol was reviewed and recommended for deferral during the RAC meeting of March 1–2, 1993, by a vote of 16 in favor, 1 opposed, and 1 abstention. Approval of the protocol was deferred until the investigators submit the following data for full RAC review: (1) Generation of cytotoxic T lymphocytes (CTL) for human peripheral blood lymphocyte from tumor-bearing patients in response to transduced autologous tumor, (2) quantitative correlation between Class I antigen expression and the amount of human gamma-interferon (IFN), and (3) quantitative correlation between the number of CTLs generated per unit of human gamma-IFN.

In a letter dated April 9, 1993, Dr. Seigler resubmitted the human gene therapy protocol with additional data to the Recombinant DNA Advisory Committee for formal review and approval. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 20 in favor, 0 opposed, and no abstentions. The following section may be added to Appendix D:

"Appendix D–XLVII

"Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina, may conduct experiments on 20 patients with disseminated malignant melanoma. Autologous tumor cells will be transduced with a retrovirus vector, pHuγ–IFN, that contains the gene encoding human γ–IFN. Following lethal irradiation, the transduced cells will be readministered to patients for the purpose of generating cytotoxin T cells that are tumor specific along with the up-regulation of Class I major histocompatibility antigens. Patients will be monitored for clinical regression of tumors and generation of tumor-specific cytotoxic T lymphocytes."

I accept this recommendation, and Appendix D–XLVII of the NIH Guidelines will be added accordingly.

B. Addition of Appendix D–XLVIII to the NIH Guidelines

In a letter dated April 10, 1993, Drs. Stefan Karlsson and Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, and Dr. Donald B. Kohn of the Childrens Hospital of Los Angeles, Los Angeles, California, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 14 in favor, 0 opposed, and 4 abstentions. The following section may be added to Appendix D:

"Appendix D–XLVIII

"Drs. Stefan Karlsson and Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, and Dr. Donald B. Kohn of the Childrens Hospital of Los Angeles, Los Angeles, California, may conduct experiments on 10 patients with Gaucher disease. CD34(+) hematopoietic stem cells will be isolated from bone marrow or from peripheral blood treated with

granulocyte-colony stimulating factor. CD34(+) cells will be transduced with a retrovirus vector, G1Gc, containing cDNA encoding human glucocerebrosidase and administered intravenously. Patients will be monitored for toxicity and glucocerebrosidase expression."

I accept this recommendation, and Appendix D–XLVIII of the NIH Guidelines will be added accordingly.

C. Addition of Appendix D–XLIX to the NIH Guidelines

In a letter dated April 9, 1993, Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Molecular Genetic Intervention for AIDS—Effects of a Transdominant Negative Form of Rev. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 14 in favor, 0 opposed, and 4 abstentions. Approval of the protocol was contingent on the submission of a revised Informed Consent document including the following: (1) A recommendation that contraception be used by males as well as females, and (2) a request for autopsy in the event of death, and (3) an explanation of long-term patient follow-up.

On June 15, 1993, Dr. Nabel submitted the modified Informed Consent document. The modified Informed Consent document was reviewed by a primary reviewer of the protocol, and it was determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D–XLIX

"Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 12 patients with AIDS to be divided into 4 experimental groups. CD4(+) lymphocytes will be isolated from peripheral blood and transduced with Rev M10, a transdominant inhibitory mutant of the *rev* gene of the human immunodeficiency virus (HIV). Transduction of the *rev* mutant will be mediated either by the retrovirus vector, PLJ–cREV M10, or a plasmid DNA/liposome complex. Patients will be monitored for survival of the transduced CD4(+) cells by polymerase chain reaction and whether Rev M10 can confer protection against HIV infection to CD4(+) cells."

I accept this recommendation, and Appendix D–XLIX of the NIH Guidelines will be added accordingly.

D. Addition of Appendix D–L to the NIH Guidelines

In a letter dated April 9, 1993, Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Immunotherapy for Cancer by Direct Gene Transfer into Tumors. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 19 in favor, 0 opposed, and 1 abstention.

No restrictions were placed on the types of tumors to be treated. Approval of the protocol was contingent on the review and approval of materials submitted in response to the following stipulations: (1) A revised patient eligibility section including the provision that patients who are eligible for catheter delivery of cationic liposomes must have a performance status of 0–1, and (2) a revised Informed Consent document including a recommendation that male/female patients use contraception and females not be pregnant or plan to become pregnant while participating in the study, an explanation of long-term follow-up, and a request for autopsy in the event of death.

On June 15, 1993, Dr. Nabel submitted the modified patient eligibility section and a modified Informed Consent document. These documents were reviewed by the primary reviewers of the protocol, and it was determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D–L

"Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 24 patients with advanced cancer. Patients will undergo *in vivo* transduction with DNA/liposome complexes containing genes encoding the HLA–B7 histocompatibility antigen and beta-2 microglobulin in a non-viral plasmid. These DNA/liposome complexes will be administered either by intratumoral injection or catheter delivery. Patients will be monitored for enhanced immune responses against tumor cells, and safe and effective doses will be determined."

I accept this recommendation, and Appendix D–L of the NIH Guidelines will be added accordingly.

E. Addition of Appendix D–LI to the NIH Guidelines

In a letter dated March 26, 1993, Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania, indicated his intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Gene Therapy for Gaucher Disease: *Ex Vivo* Gene Transfer and Autologous Transplantation of CD34(+) Cells. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 15 in favor, 0 opposed, and 4 abstentions. Approval of the protocol was contingent on review and approval of materials submitted in response to the following stipulations: (1) A maximum of 5 patients will be entered into the study, (2) patients will not receive cyclophosphamide ablation of bone marrow, (3) patients who demonstrate antibodies against glucocerebrosidase (GC) will not be eligible for this protocol, (4) GC enzyme replacement therapy will not be discontinued until cytochemical evidence of engraftment is demonstrated, and (5) the Informed Consent document will be revised to include a recommendation that male/female patients use contraception and females not be pregnant or plan to become pregnant while participating in the study, an explanation of long-term follow-up, a request for autopsy in the event of death, patient protection from media inquiries, and a statement informing the patients that although they may receive no direct benefit from the protocol, knowledge may be gained that will benefit others.

On June 11, 1993, Dr. Barranger submitted a modified protocol and Informed Consent document. These documents were reviewed by the primary reviewers of the protocol, and it was determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D–LI

"Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct experiments on 5 patients with Gaucher disease. The CD34(+) hematopoietic stem cells will be isolated from peripheral blood and transduced *in vitro* with the retrovirus

vector, N2-Sv-GC, encoding the glucocerebrosidase (GC) enzyme. Following reinfusion of the transduced cells, patients will be monitored by PCR analysis for GC expression in peripheral blood leukocytes. Patients currently receiving GC replacement therapy and who demonstrate clinical responsiveness will be withdrawn from exogenous GC therapy. Patients not previously treated with exogenous GC, will be monitored for clinical reversal of lipid storage symptoms."

I accept this recommendation, and Appendix D–LI of the NIH Guidelines will be added accordingly.

F. Addition of Appendix D–LII to the NIH Guidelines

In a letter dated July 20, 1992, Drs. Robert Walker and R. Michael Blaese, National Institutes of Health, Bethesda, Maryland, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Study of the Safety and Survival of the Adoptive Transfer of Genetically Marked Syngeneic Lymphocytes in Human Immunodeficiency Virus (HIV) Infected Identical Twins. This request was published for comment in the **Federal Register** of August 19, 1992 (57 FR 37680).

The protocol was reviewed and recommended for approval during the RAC meeting of September 14–15, 1992, by a vote of 19 in favor, 0 opposed, and no abstentions. Approval of the protocol was contingent on the following stipulations: (1) The investigators may use the vector supernatants currently in storage; however, any future vector preparations will be tested by long-term culturing of the packaging line following vector supernatant harvest, (2) the Informed Consent document should be divided into two separate documents, one for gene marking and the other for the therapeutic aspects, and (3) the section of the donor Informed Consent document describing the lymphopheresis procedure should be moved before the section describing the required travel schedule.

On July 15, 1993, Dr. Walker submitted a modified Informed Consent document. This document was reviewed by the primary reviewers of the protocol, and it was determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D–LII

"Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 12 HIV-infected patients who have a seronegative identical twin. CD4(+) and CD8(+) cells will be isolated from the seronegative twin and induced to polyclonal proliferation with anti-CD3 and interleukin-2. The enriched population of cells will be transduced with either LNL6 or G1Na, which contain the neo^R gene. The transduced cells will be expanded in tissue culture and administered to the HIV-infected twin. Patients will be monitored for immune function and the presence of marked cells."

I accept this recommendation, and Appendix D–LII of the NIH Guidelines will be added accordingly.

G. Addition of Appendix D–LII to the NIH Guidelines

In a letter dated April 5, 1993, Dr. Corey Raffel of the Childrens Hospital Los Angeles, Los Angeles, California, and Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with *In Vivo* Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene. This request was published for comment in the **Federal Register** of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 19 in favor, 0 opposed, and no abstentions. Approval of the protocol was contingent on the following stipulation: (1) The investigators must submit a document that will inform children of the experimental procedures and associated risks.

On July 13, 1993, Dr. Raffel submitted the document. This document was reviewed by two RAC members. Additional changes were requested, and the investigator incorporated those changes. It was determined that the revised document met the request of the RAC. The following section may be added to Appendix D:

"Appendix D–LIII

"Dr. Cory Raffel of the Childrens Hospital Los Angeles, Los Angeles, California, and Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa, may conduct experiments on 30 patients between 2 and 18 years of age with recurrent malignant astrocytoma. Fifteen patients will be accrued into this study initially. If at least one patient responds to therapy, an additional 14 patients will be treated. Patients with either surgically accessible or non-accessible tumors will be treated with the vector producing cell line (PA317) carrying the retrovirus vector, G1TkSvNa. This vector will transduce tumor cells *in vivo* with the *Herpes simplex* thymidine kinase (HS-tk) gene that renders the cells sensitive to killing by ganciclovir. Surgically accessible patients will undergo surgical debulking of their tumor followed by repeated administration of the HS-tk vector producer cells into the tumor bed. Children with unresectable tumors will undergo stereotaxic injection of vector producer cells into tumors."

I accept this recommendation, and Appendix D–LIII of the NIH Guidelines will be added accordingly.

H. Addition of Appendix D–LIV to the NIH Guidelines

On April 9, 1993, a human gene therapy protocol was submitted for Dr. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dr. Dennis A. Casciato of the University of California, Los Angeles, California, by Dr. Bruce Merchant, Viagene, Inc., San Diego, California (sponsor of the protocol), to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Preliminary Study to Evaluate the Safety and Biologic Effects of Murine Retroviral Vector Encoding HIV–1 Genes [HIV–IT(V)] in Asymptomatic Subjects Infected with HIV–1. This request was published for comment in the **Federal Register** of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 16 in favor, 0 opposed, and 2 abstentions. Approval of the protocol was contingent on the following stipulations: (1) The Informed Consent document must include a request for autopsy, and (2) the formulation of the excipient that will be used for the retrovirus vector preparation must be submitted for review.

On June 11, 1993, Dr. Donald Longenecker of Viagene, Inc., submitted the formulation of the vector preparation; and on June 23, 1993, Dr. Rose Hermanson of Viagene, Inc., submitted the revised Informed Consent document. These documents were reviewed by two RAC members, and it was determined that it meets the request of the RAC. The following section may be added to Appendix D:

"Appendix D–LIV

"Dr. Jeffrey E. Galpin of the University of Southern California, Los

Angeles, California and Dr. Dennis A. Casciato of the University of California, Los Angeles, California, may conduct experiments on 15 HIV(+) asymptomatic patients. Patients will receive 3 monthly intramuscular injections of the retrovirus vector (N2IIIBenv) encoding the HIV-1 IIIB envelope protein. Patients will be monitored for acute toxicity, CD4 levels, HIV-specific CTL responses, and viral burdens."

I accept this recommendation, and Appendix D–LIV of the NIH Guidelines will be added accordingly.

I. Addition of Appendix D–LV to the NIH Guidelines

In a letter dated March 8, 1993, Dr. Arthur Bank of Columbia University, New York, New York, indicated the intention of Drs. Charles Hesdorffer and Karen Antman of the Columbia University, College of Physicians and Surgeons, New York, New York, to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Human Multi-Drug Resistance (MDR) Gene Transfer in Patients with Advanced Cancer. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

The protocol was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 11 in favor, 5 opposed, and 3 abstentions. Approval of the protocol was contingent on the following stipulations: (1) submission of data demonstrating the transduction efficiency of human CD34(+) cells, and (2) providing a description of the assays that will be performed on the clinical grade supernatant.

On June 15, 1993, Dr. Charles Hesdorffer submitted the transduction efficiency data and the assay description for the clinical grade supernatant. These documents were reviewed by the primary reviewers of the protocol. It was determined that the revised document met the request of the RAC. The following section may be added to Appendix D:

"Appendix D–LV

"Drs. Charles Hesdorffer and Karen Antman of Columbia University College of Physicians and Surgeons, New York, New York, may conduct experiments on 20 patients with advanced breast, ovary, and brain cancer. CD34(+) hematopoietic stem cells will be isolated from bone marrow, transduced with the retrovirus vector, PHaMDR1/A, and readministered to patients. Patients will be monitored for the presence and expression of the MDR–1 gene. The investigators will determine whether MDR–1 expression increases following chemotherapy."

I accept this recommendation, and Appendix D–LV of the NIH Guidelines will be added accordingly.

J. Addition of Appendix D–LVI to the NIH Guidelines

In a letter dated January 8, 1993, Ms. Arvilla L. Trag of Virogenetics Corporation, Troy, New York, submitted a request on behalf of Dr. Enzo Paoletti for reduction in physical containment from Biosafety Level 2 to Biosafety Level 1 for three poxvirus vectors: NYVAC, ALVAC, and TROVAC. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676). NYVAC is derived from the Copenhagen strain of vaccinia virus in which 18 viral genes have been deleted. ALVAC is derived from an attenuated strain of canarypox virus. TROVAC is derived from an attenuated strain of fowlpox virus. These vectors are proposed for use in the production of animal vaccines.

This request was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 15 in favor, 0 opposed, and 1 abstention. The following section may be added to Appendix D:

"Appendix D–LVI

"Dr. Enzo Paoletti of Virogenetics Corporation, Troy, New York, may conduct experiments with poxvirus vectors NYVAC, ALVAC, and TROVAC at Biosafety Level 1."

I accept this recommendation, and Appendix D–LVI of the NIH Guidelines will be added accordingly.

K. Amendment to Section IV–C–3–c of the NIH Guidelines Regarding the Responsibility of the Office of Recombinant DNA Activities

In a letter dated April 5, 1993, Ms. Debra Wilson of the National Institutes of Health submitted a request that Section IV–C–3–c be amended to delete the requirement that the Office of Recombinant DNA Activities (ORDA) publish the Recombinant DNA Technical Bulletin, and to add the requirement that ORDA will serve as the focal point for data management of the NIH-approved human gene transfer protocols.

Section IV–C–3–c currently reads that the Office of Recombinant DNA Activities shall be responsible for the following:

"IV–C–3–c. Publishing the Recombinant DNA Technical Bulletin * * *."

Section IV–C–3–c proposed wording reads that the Office of Recombinant DNA Activities shall be responsible for the following:

"IV–C–3–c. Serve as the focal point for data management of NIH-approved human gene transfer protocols as required in the Reporting Requirements section of the Points to Consider."

This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

The request was reviewed and recommended for approval during the RAC meeting of June 7–8, 1993, by a vote of 16 in favor, 0 opposed, and 2 absentions. Section IV–C–3 may be amended to read that the Office of Recombinant DNA Activities shall be responsible for the following:

"IV–C–3–c. Serve as the focal point for data management of NIH-approved human gene transfer protocols as required in the Reporting Requirements section of the Points to Consider."

I accept this recommendation, and Section IV–C–3–c of the NIH Guidelines will be amended accordingly.

L. Amendment to Section III–A–4 of the NIH Guidelines and the Points To Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects

During the March 1–2, 1993, Recombinant DNA Advisory Committee meeting, Dr. Robertson Parkman requested that the terms subject and subjects be amended to read subject(s). Section III–A–4 currently reads:

"III–A–4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects [21] * * *."

Throughout the Points to Consider document the terms subject and subjects are used. This amendment will clarify Section III–A–4 and Points to Consider. This request was published for comment in the Federal Register of May 4, 1993 (58 FR 26676).

During the June 7–8, 1993, meeting, the committee reviewed the request. The committee recommended changing the term "subject(s)" to "one or more human subjects." The recommendation was approved by a vote of 20 in favor, 0 opposed, and no abstentions. Section III–A–4 may be amended to read:

"III–A–4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subject [21] * * *."

The term "subject" and "subjects" will be changed throughout the NIH Guidelines to "one or more human subjects."

I accept this recommendation, and Section III–A–4 of the NIH Guidelines and the Points to Consider will be amended accordingly.

II. Summary of Actions

A. Addition of Appendix D–XLVII to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Hilliard F. Seigler of Duke University Medical Center, Durham, North Carolina, may conduct experiments on 20 patients with disseminated malignant melanoma. Autologous tumor cells will be transduced with a retroviral vector, pHuγ–IFN, that contains the gene encoding human γ–IFN. Following lethal irradiation, the transduced cells will be readministered to patients for the purpose of generating cytotoxin T cells that are tumor specific along with the up-regulation of Class I major histocompatibility antigens. Patients will be monitored for clinical regression of tumors and generation of tumor-specific cytotoxic T lymphocytes."

B. Addition of Appendix D–XLVIII to the NIH Guidelines

The following section is added to Appendix D:

"Drs. Stefan Karlsson and Cynthia Dunbar of the National Institutes of Health, Bethesda, Maryland, and Dr. Donald B. Kohn of the Childrens Hospital of Los Angeles, Los Angeles, California, may conduct experiments on 10 patients with Gaucher disease. CD34(+) hematopoietic stem cells will be isolated from bone marrow or from peripheral blood treated with granulocyte-colony stimulating factor. CD34(+) cells will be transduced with a retrovirus vector, G1Gc, containing cDNA encoding human glucocerebrosidase and administered intravenously. Patients will be monitored for toxicity and glucocerebrosidase expression."

C. Addition of Appendix D–XLIX to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 12 patients with AIDS to be divided into 4 experimental groups. CD4(+) lymphocytes will be isolated from peripheral blood and transduced with Rev M10, a transdominant inhibitory mutant of the rev gene of the human immunodeficiency virus (HIV). Transduction of the rev mutant will be mediated either by the retrovirus vector, PLJ-cREV M10, or a plasmid DNA/liposome complex. Patients will be monitored for survival of the transduced CD4(+) cells by polymerase chain reaction and whether Rev M10 can confer protection against HIV infection to CD4(+) cells."

D. Addition of Appendix D–L to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 24 patients with advanced cancer. Patients will undergo *in vivo* transduction with DNA/liposome complexes containing genes encoding the HLA–B7 hisotcompatibility antigen and beta-2 microglobulin in a non-viral plasmid. These DNA/liposome complexes will be administered either by intratumoral injection or catheter delivery. Patients will be monitored for enhanced immune responses against tumor cells, and safe and effective doses will be determined."

E. Addition of Appendix D–LI to the NIH Guidelines

The following section is added to Appendix D:

"Dr. John A. Barranger of the University of Pittsburgh, Pittsburgh, Pennsylvania, may conduct experiments on 5 patients with Gaucher disease. The CD34(+) hematopoietic stem cells will be isolated from peripheral blood and transduced *in vitro* with the retrovirus vector, N2–Sv–GC, encoding the glucocerebrosidase (GC) enzyme. Following reinfusion of the transduced cells, patients will be monitored by PCR analysis for GC expression in peripheral blood leukocytes. Patients currently receiving GC replacement therapy and who demonstrate clinical responsiveness will be withdrawn from exogenous GC therapy. Patients not previously treated with exogenous GC, will be monitored for clinical reversal of lipid storage symptoms."

F. Addition of Appendix D–LII to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Robert Walker of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 12 HIV-infected patients who have a seronegative identical twin. CD4(+) and CD8(+) cells will be isolated from the seronegative twin and induced to polyclonal proliferation with anit-CD3 and interleukin-2. The enriched population of cells will be transduced with either LNL6 or G1Na, which contain the neo^R gene. The transduced cells will be expanded in tissue culture and administered to the HIV-infected twin. Patients will be monitored for immune function and the presence of marked cells."

G. Addition to Appendix D–LIII to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Corey Raffel of the Childrens Hospital Los Angeles, California, and Dr. Kenneth Culver of Iowa Methodist Medical Center, Des Moines, Iowa, may conduct experiments on 30 patients between 2 and 18 years of age with recurrent malignant astrocytoma. Fifteen patients will be accrued into this study initially. If at least one patient responds to therapy, an additional 14 patients will be treated. Patients with either surgically accessible or non-accessible tumors will be treated with the vector producing cell line (PA317) carrying the retrovirus vector, G1TkSvNa. This vector will transduce tumor cells *in vivo* with the *Herpes simplex* thymidine kinase (HS-tk) gene that renders the cells sensitive to killing by ganciclovir. Surgically accessible patients will undergo surgical debulking of their tumor followed by repeated administration of the HS-tk vector producer cells into the tumor bed. Children with unresectable tumors will undergo stereotaxic injection of vector producer cells into tumors."

H. Addition of Appendix D–LIV to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Jeffrey E. Galpin of the University of Southern California, Los Angeles, California, and Dr. Dennis A. Casciato of the University of California, Los Angeles, California, may conduct experiments on 15 HIV(+) asymptomatic patients. Patients will receive 3 monthly intramuscular injections of the retrovirus vector (N2IIIBenv) encoding the HIV–1 IIIB envelope protein. Patients will be monitored for acute toxicity, CD4 levels, HIV-specific CTL responses, and viral burdens."

I. Addition of Appendix D–LV to the NIH Guidelines

The following section is added to Appendix D:

"Drs. Charles Hesdorffer and Karen Antman of Columbia University College of Physicians and Surgeons, New York, New York, may conduct experiments on 20 patients with advanced breast, ovary, and brain cancer. CD34(+) hematopoietic stem cells will be isolated from bone marrow, transduced

with the retrovirus vector, PHaMDR1/A, and readministered to patients. Patients will be monitored for the presence and expression of the MDR–1 gene. The investigators will determine whether MDR–1 expression increases following chemotherapy."

J. Addition of Appendix D–LVI to the NIH Guidelines

The following section is added to Appendix D:

"Dr. Enzo Paoletti of Virogenetics Corporation, Troy, New York, may conduct experiments with poxvirus vectors NYVAC, ALVAC, and TROVAC at Biosafety Level 1."

K. Amendment to Section IV–C–3–c of the NIH Guidelines Regarding the Responsibility of the Office of Recombinant DNA Activities

Section IV–C–3–c will read as follows:

"IV–C–3–c. Serve as the focal point for data management of NIH-approved human gene transfer protocols as required in the Reporting Requirements section of the Points to Consider."

L. Amendment to Section III–A–4 of the NIH Guidelines and the Points To Consider in the Design and Submission of Protocol for the Transfer of Recombinant DNA Into the Genome of Human Subjects

Section III–A–4 will read as follows:

"III–A–4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects [21] * * *"

The terms "subject" and "subjects" will be changed throughout the NIH Guidelines to "one or more human subjects."

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: September 3, 1993.

Ruth L. Kirschstein,

Acting Director, National Institutes of Health.

[FR Doc. 93–22261 Filed 9–10–93; 8:45 am]

BILLING CODE 4140–01–M

federal register

Monday
October 18, 1993

Part V

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research: Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth eight actions to be taken by the Director, National Institutes of Health (NIH), under the May 7, 1986, NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Office of Science Policy and Technology Transfer, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: Today eight actions are being promulgated under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These eight proposed actions were published for comment in the **Federal Register** of February 12, 1993 (58 FR 8500) and August 18, 1993 (58 FR 44098), and reviewed and recommended for approval by the NIH Recombinant DNA Advisory Committee (RAC) at its meetings on March 1–2, 1993, and September 9–10, 1993.

I. Background Information and Decisions on Actions Under the NIH Guidelines

A. Major Amendment to Appendix D–XXVII to the NIH Guidelines

On July 9, 1993, Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, indicated their intention to submit a major amendment to their human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The request was to treat an additional 15 patients who do not have acquired immunodeficiency syndrome-related lymphoma and who are not undergoing autologous bone marrow transplantation.

The current protocol is entitled: A Phase I Study of Cellular Adoptive Immunotherapy Using Genetically Modified CD8+ HIV-Specific T Cells for HIV-Seropositive Patients Undergoing Allogeneic Bone Marrow Transplant. The initial protocal was approved by the NIH Director on April 17, 1992, and published in the **Federal Register** on April 22, 1992 (57 FR 14775).

Appendix D–XXVII currently reads:

"Dr. Philip D. Greenberg of the University of Washington, Seattle, Washington, can conduct gene transfer experiments on up to 15 HIV seropositive patients undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma to evaluate the safety and efficacy of HIV-specific cytotoxic T lymphocyte (CTL) therapy. CTL will be transduced with a retroviral vector (HyTK) encoding a gene that is a fusion product of the hygromycin phosphotransferase gene (HPH) and the herpes simplex virus thymidine kinase (HSV–TK) gene. This vector will deliver both a marker gene and a suicide gene in these T cell clones in the event that patients develop side effects as a consequence of CTL therapy. Data will be correlated over time, looking at multiple parameters of HIV disease activity. The objectives of these studies include evaluating the safety and toxicity of CTL therapy, determining the duration of in vivo survival of HIV-specific CTL clones, and determining if ganciclovir therapy can eradicate genetically modified, adoptively transferred CTL cells."

The amended protocol is entitled: Phase I Study to Evaluate the Safety of Cellular Adoptive Immunotherapy using Genetically Modified CD8+ HIV-Specific T Cells in HIV Seropositive Individuals. This request was published for comment in the **Federal Register** of August 18, 1993 (58 FR 44098).

The protocol was reviewed and recommended approval during the RAC meeting of September 9–10, 1993, by a vote of 16 in favor, 0 opposed, and 2 abstentions.

The following section may be amended as follows:

"Appendix D–XXVII.

"Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 15 human immunodeficiency virus (HIV) seropositive patients (18–45 years old) undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma and 15 HIV-seropositive patients (18–50 years old) who do not have acquired immunodeficiency syndrome (AIDS)-related lymphoma and who are not undergoing bone marrow transplantation to evaluate the safety and efficacy of HIV-specific cytotoxic T lymphocyte (CTL) therapy. CTL will be transduced with a retroviral vector (HyTK) encoding a gene that is a fusion product of the hygromycin phosphotransferase gene (HPH) and the herpes simplex virus thymidine kinase (HSV–TK) gene. This vector will deliver both a marker gene and an ablatable gene in these T cell clones in the event that patients develop side effects as a consequence of CTL therapy. Data will be correlated over time, looking at multiple parameters of HIV disease activity. The objectives of these studies include evaluating the safety and toxicity of CTL therapy, determining the duration of in vivo survival of HIV-specific CTL clones, and determining if ganciclovir therapy can eradicate genetically modified, adoptively transferred CTL cells."

I accept this recommendation, and Appendix D–XXVII of the NIH Guidelines will be amended accordingly.

B. Addition of Appendix D–LVII to the NIH Guidelines

In a letter dated December 17, 1992, Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina, indicated their intention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus: A Phase I Trial in the Nasal Cavity. This request was published for comment in the **Federal Register** of February 12, 1993 (58 FR 8500).

The protocol was reviewed and recommended for approval during the RAC meeting of March 1–2, 1993, by a vote of 16 in favor, 0 opposed, and 1 abstention. Approval of this protocol was contingent on the following stipulation: the investigators must submit a letter of resolution regarding the correct sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

On June 25, 1993, Dr. James Wilson of the University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, provided information to fulfill the stipulation requirement. On September 10, 1993, Dr. Boucher provided additional materials as requested by the primary reviewers. These materials were reviewed by primary reviewers of the protocol, and it was determined that the stipulations of the RAC were met. The following section may be added to Appendix D:

"Appendix D–LVII.

"Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina, may conduct experiments on 9 patients (18 years old or greater) with cystic fibrosis to test for the safety and efficacy of an El-deleted recombinant adenovirus

containing the cystic fibrosis transmembrane conductance regulator (CFTR) cDNA, Ad.CB–CFTR. A single dose of 10^8, 3×10^9 or 10^{11} pfu/ml will be administered to the nasal cavity of 3 patients in each dose group. Patients will be monitored by nasal lavage and biopsy to assess safety and restoration of normal epithelial function."

I accept this recommendation, and Appendix D–LVII of the NIH Guidelines will be added accordingly.

C. Addition of Appendix D–LVIII to the NIH Guidelines

In a letter dated September 9, 1992, Dr. Joyce A. O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland, indicated her attention to submit a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Retroviral Mediated Transfer of the Human Multi-Drug Resistance Gene (MDR–1) into Hematopoietic Stem Cells During Autologous Transplantation after Intensive Chemotherapy for Breast Cancer.

During the December 3–4, 1992, Recombinant DNA Advisory Committee meeting, the protocol was deferred until the investigators submitted the following for full RAC review: (1) Data demonstrating that human CD34(+) cells can be transduced *in vitro* with the actual vector that will be used for the human clinical protocol, (2) a description of the methods that will be used to monitor gene expression in bone marrow and tumor cells, and (3) a description of the endpoint for determining bone marrow recovery, i.e., comparison of gene amplification and the rate of polymorphonuclear leukocyte recovery following taxol administration.

On July 14, 1993, Dr. O'Shaughnessy resubmitted a human gene therapy protocol for RAC review and approval. This request was published for comment in the Federal Register of August 18, 1993 (58 FR 44098).

The protocol was reviewed and recommended for approval during the RAC meeting of September 9–10, 1993 by a vote of 17 in favor, 0 opposed, and no abstentions. The following section may be added to Appendix D.

"Appendix D–LVIII.

"Dr. Joyce A. O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 18 patients (18–60 years old) with Stage IV breast cancer who have achieved a partial or complete response to induction chemotherapy. This study will determine the feasibility of obtaining engraftment of CD34(+) hematopoietic stem cells transduced by a retroviral vector, G1MD, and expressing a cDNA for human multi-drug resistance–1 (MDR–1) gene following high dose chemotherapy, and whether the transduced MDR–1 gene confers drug resistance to hematopoietic cells and functions as an in vivo dominant selectable marker. Patients will be monitored for evidence of myeloprotection and presence of the transduced MDR–1 gene."

I accept this recommendation, and Appendix D–LVIII of the NIH Guidelines will be added accordingly.

D. Addition of Appendix D–LIX to the NIH Guidelines

On July 12, 1993, Drs. Larry E. Kun, R.A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the National Institutes of Health, Bethesda, Maryland, submitted a human gene therapy protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Gene Therapy for Recurrent Pediatric Brain Tumors. This request was published for comment in the Federal Register of August 18, 1993 (58 FR 44098).

The protocol was reviewed and recommended for approval during the RAC meeting of September 9–10, 1993 by a vote of 17 in favor, 0 opposed, and no abstentions. The RAC requested that the Office of Recombinant DNA Activities send a letter to the Institutional Review Board strongly recommending that the Informed Consent document should be separated into two separate documents: (1) A patient assent form, and (2) a guardian consent form. On September 21, 1993, the Office of Recombinant DNA Activities sent a letter to the Institutional Review Board at the St. Jude Children's Research Hospital and the LeBonheur Children's Medical Center regarding the Committee's recommendations. The following section may be added to Appendix D:

"Appendix D–LIX.

"Drs. Larry E. Kun, R.A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patients (3–21 years old) with progressive or recurrent malignant supratentorial tumors resistant to standard therapies. Mouse cells producing the retroviral vector containing the herpes simplex thymidine kinase gene (G1TKSVNa) will be instilled into the tumor areas via multiple stereotactically placed cannulas. Patients will be treated with ganciclovir to eliminate cells expressing the transduced gene. Patients will be monitored for central nervous system, hematologic, renal or other toxicities, and for anti-tumor responses by magnetic resonance imaging studies.

I accept this recommendation, and Appendix D–LIX of the NIH Guidelines will be added accordingly.

E. Addition of Appendix D–LX to the NIH Guidelines Regarding Semliki Forest Virus

Dr. Gary F. Temple of Life Technologies, Inc., Gaithersburg, Maryland, submitted a request for a reduction in physical containment from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus (SFV) vector expression system.

During the September 14–15, 1992, Recombinant DNA Advisory Committee meeting, the request was deferred so that the investigators could acquire data regarding the following: (1) The frequency of recombination that produces replication-competent virus, using cell numbers analogous to the laboratory setting (e.g., 1×10^9 cells), and (2) acquire data regarding the frequency of seropositivity among personnel previously exposed to SFV.

During the June 7–8, 1993, Recombinant DNA Advisory Committee meeting, the request was deferred until the investigators returned to the Committee with the following: (1) A product information sheet informing customers of the potential health risks associated with the expression system, standard methods to be used for virus inactivation, a helper virus assay to detect SFV, and a description of symptoms and procedures to be followed in the event that SFV infection occurs in a laboratory worker (including methods to prevent transfer to insect vectors and environmental spread); and (2) SFV inactivation data.

In a letter dated February 8, 1993, Dr. Temple resubmitted a request for a reduction in physical containment from Biosafety Level 3 to Biosafety Level 2 for a SFV vector expression system. This request was published for comment in the Federal Register on August 18, 1993 (58 FR 44098).

During the September 9–10, 1993, Recombinant DNA Advisory Committee meeting, this request was reviewed and recommended for approval by a vote of 13 in favor, 2 opposed, and 1 abstention. The physical containment for Life Technologies, Inc., SFV vector expression system may be reduced from Biosafety Level 3 to Biosafety Level 2.

Approval of the request is contingent on the following stipulation: (1) Principal investigators using this expression system must obtain signatures from all laboratory personnel certifying that they have been informed of the possible risks associated with this vector expression system and that they read the Product Information booklet. The following section may be added to Appendix D:

"Appendix D–LX.

"The physical containment level may be reduced from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus (SFV) vector expression system of Life Technologies, Inc., Gaithersburg, Maryland."

I accept this recommendation, and Appendix D–LX of the NIH Guidelines will be added accordingly.

F. Amendment to Section III and Appendix D of the NIH Guidelines Regarding Actions Taken Under the Guidelines

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, requested an amendment to Section III and Appendix D to eliminate the requirement for publication of Appendix D (Actions Taken Under the Guidelines) in the Federal Register and to allow distribution of these actions by the Office of Recombinant DNA Activities. This request was published for comment in the Federal Register of August 18, 1993 (58 FR 44098). Section III–A and Appendix D is proposed to read:

"Section III–A—Experiments that Require RAC Review and IBC Approval Before Initiation.

* * * Specific experiments already approved in this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

"Appendix D—Actions Taken Under the Guidelines.

"As noted in the subsection of Section IV–C–1–b–(1), the Director, NIH, may take certain actions with regard to the Guidelines after the issues have been considered by the RAC. An updated list of these actions are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

This request was reviewed and recommended for approval as proposed during the RAC meeting of September 9–10, 1993, by a vote of 16 in favor, 0 opposed, and no abstentions.

I accept this recommendation, and Appendix D of the NIH Guidelines will be amended accordingly.

G. Amendment to the Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC of the Points to Consider/NIH Guidelines

Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, requested an amendment to the Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC Federal Register, February 18, 1993, page 9104). These amendments will establish consistency in the submission of human gene transfer protocols for RAC review and require that principal investigators focus their oral responses to the RAC's questions and comments. This request was published for comment in the Federal Register of August 18, 1993 (58 FR 44098). The Title and Section I was proposed to read:

"Guidelines for the Submission of Human Gene Transfer Protocols for Review by the Recombinant DNA Advisory Committee

"I. Investigator Submitted Material:

"Written proposals must be submitted in the following order: (1) Scientific abstract—1 page; (2) non-technical abstract—1 page; (3) Institutional Biosafety Committee and Institutional Review Board approvals; (4) Points to Consider—5 pages; (5) protocol—20 pages excluding appendices; (7) Informed Consent Document—approved by the Institutional Review Board; (8) appendices including tables, figures, and manuscripts; and (9) Curricula vitae—2 pages in Biographical sketch format. When a proposal has been submitted previously, there should be a short section (≤200 words) immediately following the abstracts that summarizes the major revisions since the last review. Data provided * * *.

"* * * written responses (including critical data in response to the primary reviewers' comments) must be submitted by the Principal Investigators to the Office of Recombinant DNA Activities ≥2 weeks before the RAC meeting.

"Oral Responses to the RAC. Principal Investigators must limit their oral responses to the RAC only to those questions that are raised during the meeting. Oral presentations of previously submitted material and/or critical data that was not submitted ≥2 weeks prior to the RAC meeting is prohibited."

This request was reviewed and recommended for approval as proposed during the RAC meeting of September 9–10, 1993, by a vote of 16 in favor, 1 opposed, and no abstentions.

I accept this recommendation, and the title and section I to the Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC (Federal Register, February 18, 1993, page 9104) of the NIH Guidelines will be amended accordingly.

H. Amendments to Sections III, IV, V and Appendix C and F of the NIH Guidelines Regarding the Cloning of Toxin Molecules

In a letter dated July 28, 1993, Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland, requested amendments to Sections III, IV, and V, and Appendices C and F regarding the review process for experiments involving the cloning of toxin molecules. These amendments will establish a new category of review entitled: Experiments that Require NIH (Office of Recombinant DNA Activities/ORDA) and Institutional Biosafety Committee (IBC) Approval Before Initiation. Under this new category of review, experiments involving the cloning of toxin molecules that are lethal for vertebrates at an LD_{50} of <100 nanograms per kilogram of body weight will be reviewed by NIH (ORDA) in consultation with ad hoc toxin experts. This request was published for comment in the Federal Register of August 18, 1993 (58 FR 44098). Sections III, IV, V and Appendix C and F is proposed to read:

"Section III. Guidelines for Covered Experiments.

"Part III discusses experiments involving recombinant DNA. These experiments have been divided into five classes:

"III–A. Experiments which require specific RAC review and NIH and IBC approval before initiation of the experiment;

"III–B. Experiments which require NIH (Office of Recombinant DNA Activities/ORDA) and Institutional Biosafety Committee (IBC) approval before initiation of the experiment;

"III–C. Experiments which require IBC approval before initiation of the experiment;

"III–D. Experiments which require IBC notification at the time of the experiment;

"III–E. Experiments which are exempt from the procedures of the Guidelines.

"IF AN EXPERIMENT FALLS INTO BOTH CLASS III–A AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS III–A MUST BE FOLLOWED. If an experiment falls

into Class III–E and into either Class III–C or III–D as well, it can be considered exempt from the requirements of the Guidelines. Changes * * *"

Section III–A–I will be moved to a new Section III–B–1. New Section III–B is proposed to read:

"Section III–B–Experiments That Require NIH (ORDA) and IBC Approval Before Initiation.

"Section III–B–1. Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight * * *

"Section III–B–1–(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH through ORDA. The containment conditions for such experiments will be determined by ORDA in consultation with ad hoc experts. Such experiments also require the approval of the IBC before initiation (see Section IV–C–1–b–(3)–(f)."

Sections III–A–2, III–A–3, III–A–4 will be renumbered to III–A–1, III–A–2, III–A–3 respectively. Sections III–B, III–C, and III–D will be renumbered to III–C, III–D, and III–E respectively.

The new Section III–C–2 is proposed to read:

"Section III–C–2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) is Cloned in Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.

"Section III–C–2–a. * * * Many experiments in this category are exempt from the Guidelines (see Section III–E–4) and III–E–5). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH (ORDA) approval (see Section III–B–1) or must be carried out under NIH specified conditions as described in Appendix F."

Section IV–B–5–b–(3) is proposed to read:

"Section IV–B–5–b–(3). Petition NIH (ORDA), with concurrence of the IBC for approval to conduct experiments specified in Sections III–A and III–B of the Guidelines;"

Section IV–C–1–b–(3)–(f) will be deleted which reads: "Approving the cloning of toxin genes in host-vector systems other than E. coli K–12 (see Appendix F); and]"

Section IV–C–1–b–(3)–(g) will become the new Section IV–C–1–b–(3)–(f).

The new Section IV–C–3–a is proposed to read:

"Reviewing and approving experiments involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an $LD_{50} \leq 100$ nanograms per kilogram body weight in organisms other than E. coli K–12 (see Section III–B–1 and Appendices F–I and F–II)."

Sections IV–C–3–a and IV–C–3–b will be renumbered to become Sections IV–C–3–b and IV–C–3–c respectively.

Section V–2 is proposed to read:

"* * * In the cases falling under Sections III–A through III–D, this judgment is to be reviewed and approved by the IBC * * *"

Appendix C is proposed to read:

"Appendix C. Exemptions Under Section III–D–5.

"* * * Appendix C–I. Recombinant DNA in Tissue Culture * * *

"* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents, and (iv) experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

"* * * Appendix C–II. Experiments Involving E. coli K–12 Host-Vector Systems * * *

"* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior IBC review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

"* * * Appendix C–III. Experiments Involving Saccharomyces Host-Vector Systems * * *

"* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

"* * * Appendix C–IV. Experiments Involving Bacillus subtilis Host-Vector Systems * * *

"* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

"* * * Appendix C–V. Extrachromosomal Elements of Gram Positive Organisms * * *

"* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F.)"

Appendix F is proposed to read:

"Appendix F. Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates.

"Appendix F–I. General Information.

"Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of <100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin) are covered under Section III–B–1 of the Guidelines and require NIH (ORDA) and IBC approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight.

Experiments involving genes coding for toxin molecules with an LD_{50} of <100 micrograms and >100 nanograms per kilogram body weight require registration with ORDA and IBC approval prior to initiating the experiments. A list of toxin molecules classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxin molecules not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with ad hoc experts, prior to inclusion of the molecules on the list (see Section IV–C–1–b–(2)–(e)) * * *.

"Appendix F–III. Containment Conditions for Cloning Toxin Molecule Genes in Organisms Other Than E. coli K–12.

"Requests involving the cloning of genes coding for molecules toxic for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight in host-vector systems other than E. coli K–12 will be evaluated by NIH (ORDA) in consultation with ad hoc toxin experts (see Sections III–B and IV–C–1–b–(3)–(f).

"Appendix F–IV. Specific Approvals.

"An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

Appendix F–IV–A through Appendix F–IV–K would be deleted. [A list of these specific approvals will be maintained in ORDA.]

This request was reviewed and recommended for approval as proposed during the RAC meeting of September 9–10, 1993, by a vote of 16 in favor, 0 opposed, and no abstentions.

I accept this recommendation, and Sections III, IV, V and Appendix C and F of the NIH Guidelines will be amended accordingly.

II. Summary of Actions

A. Major Amendment to Appendix D–XXVII to the NIH Guidelines

Appendix D–XXVII will read as follows:

"Appendix D–XXVII.

"Drs. Philip Greenberg and Stanley R. Riddell of the Fred Hutchinson Cancer Research Center, Seattle, Washington, may conduct gene transfer experiments on 15 human immunodeficiency virus (HIV) seropositive patients (18–45 years old) undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma and 15 HIV-seropositive patients (18–50 years old) who do not have acquired immunodeficiency syndrome (AIDS)-related lymphoma and who are not undergoing bone marrow transplantation to evaluate the safety and efficacy of HIV-specific cytotoxic T lymphocyte (CTL) therapy. CTL will be transduced with a retroviral vector (HyTK) encoding a gene that is a fusion product of the hygromycin phosphotransferase gene (HPH) and the herpes simplex virus thymidine kinase (HSV–TK) gene. This vector will deliver both a marker gene and an ablatable gene in these T cell clones in the event that patients develop side effects as a consequence of CTL therapy. Data will be correlated over time, looking at multiple parameters of HIV disease activity. The objectives of these studies include evaluating the safety and toxicity of CTL therapy, determining the duration of in vivo survival of HIV-specific CTL clones, and determining if ganciclovir therapy can eradicate genetically modified, adoptively transferred CTL cells."

B. Addition of Appendix D–LVII to the NIH Guidelines

The following section is added to Appendix D:

"Appendix D–LVII.

"Drs. Richard C. Boucher and Michael R. Knowles of the University of North Carolina, Chapel Hill, North Carolina, may conduct experiments on 9 patients (18 years old or greater) with cystic fibrosis to test for the safety and efficacy of and E1-deleted recombinant adenovirus containing the cystic fibrosis transmembrane conductance regulator (CFTR) cDNA, Ad.CB–CFTR. A single dose of 10^6, 3×10^6 or 10^{11} pfu/ml will be administered to the nasal cavity of 3 patients in each dose group. Patients will be monitored by nasal lavage and biopsy to assess safety and restoration of normal epithelial function."

C. Addition of Appendix D–LVIII to the NIH Guidelines

The following section is added to Appendix D:

"Appendix D–LVIII.

" Dr. Joyce A. O'Shaughnessy of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 18 patients (18–60 years old) with Stage IV breast cancer who have achieved a partial or complete response to induction chemotherapy. This study will determine the feasibility of obtaining engraftment of CD34(+) hematopoietic stem cells transduced by a retroviral vector, G1MD, and expressing a cDNA for the human multi-drug resistance-1 (MDR–1) gene following high dose chemotherapy, and whether the transduced MDR–1 gene confers drug resistance to hematopoietic cells and functions as in *in vivo* dominant selectable marker. Patients will be monitored for evidence of myeloprotection and presence of the transduced MDR–1 gene."

D. Addition of Appendix D–LIX to the NIH Guidelines

The following section is added to Appendix D:

"Appendix D–LIX.

"Drs. Larry E. Kun, R. A. Sanford, Malcolm Brenner, and Richard L. Heideman of St. Jude Children's Research Hospital, Memphis, Tennessee, and Dr. Edward H. Oldfield of the National Institutes of Health, Bethesda, Maryland, may conduct experiments on 6 patents (3–21 years old) with progressive or recurrent malignant supratentorial tumors resistant to standard therapies. Mouse cells producing the retroviral vector containing the herpes simplex thymidine kinase gene (G1TKSVNa) will be instilled into the tumor areas via multiple stereotactically placed cannulas. Patients will be treated with ganciclovir to eliminate cells expressing the transduced gene. Patients will be monitored for central nervous system, hematologic, renal or other toxicities, and for anti-tumor responses by magnetic resonance imaging studies."

E. Addition of Appendix D–LX to the NIH Guidelines Regarding Semliki Forest Virus

The following section is added to Appendix D:

"Appendix D–LX.

"The physical containment level may be reduced from Biosafety Level 3 to Biosafety Level 2 for a Semliki Forest Virus (SFV) vector expression system of Life Technologies, Inc., Gaithersburg, Maryland."

F. Amendment to Section III and Appendix D of the NIH Guidelines Regarding Actions Taken Under the Guidelines

Section III–A and Appendix D will read as follows:

"Section III–A—Experiments that Require RAC Review and IBC Approval Before Initiation.

"* * * Specific experiments already approved in this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

"Appendix D—Actions Taken Under the Guidelines.

"As noted in the subsection of Section IV–C–1–b–(1), the Director, NIH, may take certain actions with regard to the Guidelines after the issues have been considered by the RAC. An updated list of these actions are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

G. Amendment to the Guidelines for the Submission of Human Gene Transfer/ Therapy Protocols for Review by the RAC of the Points to Consider/NIH Guidelines

The Title and Section I of the Guidelines for the Submission of Human Gene Transfer/Therapy Protocols for Review by the RAC (Federal Register, February 18, 1993, page 9104) will read as follows:

"Guidelines for the Submission of Human Gene Transfer Protocols for Review by the Recombinant DNA Advisory Committee

"I. Investigator Submitted Material:

"Written proposals must be submitted in the following order: (1) Scientific abstract—1 page; (2) non-technical abstract—1 page; (3) Institutional Biosafety Committee and Institutional Review Board approvals; (4) Points to Consider—5 pages; (6) protocol—20 pages excluding appendices; (7) Informed Consent Document—approved by the Institutional Review Board; (8) appendices including tables, figures, and manuscripts; and (9) Curricula vitae—2 pages in Biographical sketch format. When a proposal has been submitted previously, there should be a short section (≤200 words) immediately following the abstracts that summarizes the major revisions since the last review. Data provided * * *.

"* * * written responses (including critical data in response to the primary reviewers' comments) must be submitted by the Principal Investigators to the Office of Recombinant DNA Activities ≥2 weeks before the RAC meeting.

"Oral Responses to the RAC. Principal Investigators must limit their oral responses to the RAC only to those questions that are raised during the meeting.

Oral presentations of previously submitted material and/or critical data that was not submitted ≥2 weeks prior to the RAC meeting is prohibited."

H. Amendments to Sections III, IV, V and Appendix C and F of the NIH Guidelines Regarding the Cloning of Toxin Molecules

Sections III, IV, and V, and Appendices C and F will be amended as follows:

"Section III. Guidelines for Covered Experiments.

"Part III discusses experiments involving recombinant DNA. These experiments have been divided into five classes:

"III–A. Experiments which require specific RAC review and NIH and IBC approval before initiation of the experiment;

"III–B. Experiments which require NIH (Office of Recombinant DNA Activities/ORDA) and Institutional Biosafety Committee (IBC) approval before initiation of the experiment;

"III–C. Experiments which require IBC approval before initiation of the experiment;

"III–D. Experiments which require IBC notification at the time of the experiment;

"III–E. Experiments which are exempt from the procedures of the Guidelines.

"IF AN EXPERIMENT FALLS INTO BOTH CLASS III–A AND ONE OF THE OTHER CLASSES, THE RULES PERTAINING TO CLASS III–A MUST BE FOLLOWED. If an experiment falls into Class III–E and into either Class III–C or III–D as well, it can be considered exempt from the requirements of the Guidelines. Changes * * * "

Section III–A–I will be moved to a new Section III–B–1. New Section III–B will read:

"Section III–B–Experiments That Require NIH (ORDA) and IBC Approval Before Initiation.

"Section III–B–1. Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight * * *

"Section III–B–1–(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH through ORDA. The containment conditions for such experiments will be determined by ORDA in consultation with ad hoc experts. Such experiments also require the approval of the IBC before initiation (see Section IV–C–1–b–(3)–(f)."

Sections III–A–2, III–A–3, III–A–4 will be renumbered to III–A–1, III–A–2, III–A–3 respectively. Sections III–B, III–C, III–D will be renumbered to III–C, III–D, and III–E respectively.

The new Section III–C–2 will read:

"Section III–C–2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents [1]) is Cloned in Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.

"Section III–C–2–a. * * * Many experiments in this category are exempt from the Guidelines (see Section III–E–4) and III–E–5). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH (ORDA) approval (see Section III–B–1) or must be carried out under NIH specified conditions as described in Appendix F."

Section IV–B–5–b–(3) will read:

"Section IV–B–5–b–(3). Petition NIH (ORDA), with concurrence of the IBC for approval to conduct experiments specified in Sections III–A and III–B of the Guidelines;"

Section IV–C–1–b–(3)–(f) will be deleted which reads: "Approving the cloning of toxin genes in host-vector systems other than E. coli K–12 (see Appendix F); and"

Section IV–C–1–b–(3)–(g) will become the new Section IV–C–1–b–(3)–(f).

The new Section IV–C–3–a will read:

"Reviewing and approving experiments involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD_{50} ≤100 nanograms per kilogram body weight in organisms other than E. coli K–12 (see Section III–B–1 and Appendices F–I and F–II)."

Sections IV–C–3–a and IV–C–3–b will be renumbered to become Sections IV–C–3–b and IV–C–3–c respectively.

Section V–2 will read:

"* * * In the cases falling under Sections III–A through III–D, this judgment is to be reviewed and approved by the IBC * * *"

Appendix C will read:

"Appendix C. Exemptions Under Section III–D–5.

"* * * Appendix C–I. Recombinant DNA in Tissue Culture * * *

"* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (1) experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents, and (iv) experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

"* * * Appendix C–II. Experiments Involving E. coli K–12 Host-Vector Systems * * *

"* * * Exceptions. The following categories are not exempt from the NIH

Guidelines: (i) experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior IBC review and approval, (iv) large-scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

"* * * Appendix C–III. Experiments Involving Saccharomyces Host-Vector Systems * * *

"* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

* * * Appendix C–IV. Experiments Involving Bacillus subtilis Host-Vector Systems * * *

* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (1) Experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms [1] or cells known to be infected with these agents may be conducted under containment conditions specified in Section III–C–2 with prior IBC review and approval, large-scale experiments (e.g., more than 10 liters of culture), and experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F).

"* * * Appendix C–V. Extrachromosomal Elements of Gram Positive Organisms * * *

* * * Exceptions. The following categories are not exempt from the NIH Guidelines: (i) Experiments described in Section III–A which require specific RAC review and NIH and IBC approval before initiation, (ii) experiments described in Section III–B which require NIH (ORDA) and IBC approval before initiation, (iii) large-scale experiments (e.g., more than 10 liters of culture), and (iv) experiments involving the cloning of toxin molecule genes in E. coli K–12 (see Appendix F.)"

Appendix F will read:

"Appendix F. Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates.

"Appendix F–I. General Information.

"Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD_{50} of <100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin) are covered under Section III–B–1 of the Guidelines and require NIH (ORDA) and IBC approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD_{50} of 100 micrograms or more per kilogram of body weight. Experiments involving genes coding for toxin molecules with an LD_{50} of <100 micrograms and >100 nanograms per kilogram body weight require registration with ORDA and IBC approval prior to initiating the experiments. A list of toxin molecules classified as to LD_{50} is available from ORDA. Testing procedures for determining toxicity of toxin molecules not on the list are available from ORDA. The results of such tests shall be forwarded to ORDA, which will consult with ad hoc experts, prior to inclusion of the molecules on the list (see Section IV–C–1–b–(2)–(e)) * * *

"Appendix F–III. Containment Conditions for Cloning Toxin Molecule Genes in Organisms Other Than *E. coli* K–12.

"Requests involving the cloning of genes coding for molecules toxic for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight in host-vector systems other than *E. coli* K–12 will be evaluated by NIH (ORDA) in consultation with *ad hoc* toxin experts (see Sections III–B and IV–C–1–b(3)–(f).

"Appendix F–IV. Specific Approvals.

"An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892."

Appendix F–IV–A through Appendix F–IV–K would be deleted. [A list of these specific approvals will be maintained in ORDA.]

III. Correction to the Notice of Actions Published in the Federal Register on September 13, 1993 (58 FR 47906)

Appendix D–XLIX should read:

"Appendix D–XLIX.

"Dr. Gary J. Nabel of the University of Michigan Medical Center, Ann Arbor, Michigan, may conduct experiments on 12 patients with AIDS to be divided into 4 experimental groups. CD4(+) lymphocytes will be isolated from peripheral blood and transduced with Rev M10, a transdominant inhibitory mutant of the *rev* gene of the human immunodeficiency virus (HIV). Transduction of the *rev* mutant will be mediated either by the retrovirus vector, PLJ-cREV M10, or by particle-mediated gene transfer of plasmid DNA. Patients will be monitored for survival of the transduced CD4(+) cells by polymerase chain reaction and whether Rev M10 can confer protection against HIV infection to CD4(+) cells."

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title and affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost-effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Effective Date: October 7, 1993.

Ruth L. Kirschstein,

Acting Director, National Institutes of Health

[FR Doc. 93–25498 Filed 10–15–93, 8:45 am]

BILLING CODE 4140–01–M

Tuesday
November 9, 1993

Part VI

Department of Health and Human Services

National Institutes of Health

Recombinant DNA; Advisory Committee Meeting and Proposed Actions Under the Guidelines; Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Public Law 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee on December 2–3, 1993. The meeting will be held at the National Institutes of Health, Building 31C, 6th Floor, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892, starting at approximately 9 a.m. on December 2, 1993, to adjournment at approximately 5 p.m. on December 3, 1993. The meeting will be open to the public to discuss Proposed Actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958) and other matters to be considered by the Committee. The Proposed Actions to be discussed will follow this notice of meeting.

Dr. Nelson A. Wivel, Director, Officer of Recombinant DNA Activities, National Institutes of Health, Building 31, room 4B11, Bethesda, Maryland 20892, Phone (301) 496–9838, FAX (301) 496–9839, will provide materials to be discussed at this meeting, roster of committee members, and substantive program information. Individuals who plan to attend and need special assistance, such as sign language interpretation or other reasonable accommodations, should contact Dr. Wivel in advance of the meeting. A summary of the meeting will be available at a later date.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: October 29, 1993.

Susan K. Feldman,

Committee Management Officer, NIH.

[FR Doc. 93–27571 Filed 11–8–93; 8:45 am]

BILLING CODE 4140–01–M

Recombinant DNA Research; Proposed Actions Under the Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of proposed actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958).

SUMMARY: This notice sets forth proposed actions to be taken under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (51 FR 16958). Interested parties are invited to submit comments concerning these proposals. These proposals will be considered by the Recombinant DNA Advisory Committee (RAC) at its meeting on December 2–3, 1993. After consideration of these proposals and comments by the RAC, the Director of the National Institutes of Health will issue decisions in accordance with the NIH Guidelines.

DATES: Comments received by November 22, 1993, will be reproduced and distributed to the RAC for consideration at its December 2–3, 1993, meeting.

ADDRESSES: Written comments and recommendations should be submitted to Dr. Nelson A. Wivel, Director, Office of Recombinant DNA Activities (ORDA), Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, or sent by FAX to 301–496–9839.

All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5 p.m.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from the Office of Recombinant DNA Activities, Building 31, room 4B11, National Institutes of Health, Bethesda, Maryland 20892, (301) 496–9838.

SUPPLEMENTARY INFORMATION: The NIH will consider the following actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules:

I. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Chang

On October 6, 1993, Dr. Alfred E. Chang of the University of Michigan Medical Center, Ann Arbor, Michigan, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the IL–4 Gene.

II. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Haubrich

On October 6, 1993, Dr. Richard Haubrich of the University of California at San Diego Treatment Center, San Diego, California (sponsored by Viagene, San Diego, California), submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: An Open Label, Phase I/II Clinical Trial to Evaluate the Safety and Biological Activity of HIV–IT(V) (HIV–1 II Benv/rev-Retroviral Vector) in HIV–1–Infected Subjects.

III. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Liu and Young

On October 7, 1993, Drs. Johnson M. Liu and Neal S. Young of the National Institutes of Health, Bethesda, Maryland, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Retroviral Medicated Gene Transfer of the Fanconi Anemia Complementation Group C Gene to Hematopoietic Progenitors of Group C Patients.

IV. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Oldfield and Ram

On October 1, 1993, Drs. Edward H. Oldfield and Zvi Ram of the National Institutes of Health, Bethesda, Maryland, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis.

V. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Schuening

On September 29, 1993, Dr. Friedrich Schuening of the Fred Hutchinson Cancer Research Center, Seattle, Washington, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Retrovirus-Mediated Transfer of the cDNA for Human Glucocerebrosidase into Peripheral Blood Repopulating Cells of Patients with Gaucher's Disease.

VI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Sorscher and Logan

On October 6, 1993, Drs. Eric J. Sorscher and James J. Logan of the University of Alabama, Birmingham, Alabama, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Gene Therapy for Cystic Fibrosis Using Cationic Liposome Mediated Gene Transfer: A Phase I Trial of Safety and Efficacy in the Nasal Airway.

VII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Sznol

On October 7, 1993, Dr. Mario Sznol of the National Institutes of Health, Bethesda, Maryland, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: A Phase I Trial of B7-Transfected Lethally Irradiated Allogeneic Melanoma Cell Lines to Induce Cell-Mediated Immunity Against Tumor-Associated Antigens Presented by HLA–A2 or HLA–A1 in Patients with Stage IV Melanoma.

VIII. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Rubin

On October 6, 1993, Dr. Joseph Rubin of the Mayo Clinic, Rochester, Minnesota (sponsored by Vical, Inc., San Diego, California), submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Phase I Study of Immunotherapy of Advanced Colorectal Carcinoma by Direct Gene Transfer into Hepatic Metastases.

IX. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Dr. Welsh

On October 4, 1993, Dr. Michael J. Welsh of the Howard Hughes Medical Institute, Iowa City, Iowa, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Adenovirus-Mediated Gene Transfer of CFTR to the Nasal Epithelium & Maxillary Sinus of Patients with Cystic Fibrosis.

X. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Sobol and Royston

On October 6, 1993, Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Immunization of Colon Carcinoma Patients with Autologous Irradiated Tumor Cells and Fibroblasts Genetically Modified to Secrete Interleukin-2 (IL–2): A Phase I Study.

XI. Addition to Appendix D of the NIH Guidelines Regarding a Human Gene Transfer Protocol/Drs. Sobol and Royston

On October 6, 1993, Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California, submitted a human gene transfer protocol to the Recombinant DNA Advisory Committee for formal review and approval. The title of this protocol is: Immunization of Glioblastoma Patients with Tumor Cells Genetically Modified to Secrete Interleukin-2 (IL–2): A Phase I Study.

XII. Report on Minor Modifications to NIH-Approved Human Gene Transfer Protocols

Dr. LeRoy Walters, Chair of the Recombinant DNA Advisory Committee, will present an update on minor modifications to NIH-approved human gene transfer protocols.

XIII. Working Group on Data Management

Dr. Brigid Leventhal, Chair of the Working Group on Data Management, will provide a summary of the reports submitted to the Office of Recombinant DNA Activities by the principal investigators of NIH-approved protocols, and make recommendations regarding actions to be taken in the event of non-reporting.

XIV. Amendments to Footnotes 21 and 22 and Section III–A–3 of the NIH Guidelines Regarding Recombinant DNA Vaccines

Dr. Stephen Straus, Chair of the Working Group on Vaccines, will present an overview of the proposed amendments to Footnotes 21 and 22 and Section III–A–3. The proposed amendments will define those categories of experiments involving the administration of recombinant DNA vaccines that are exempt from Recombinant DNA Advisory Committee review and National Institutes of Health and Institutional Biosafety Committee approval.

XV. Amendments to Sections III, IV, V of the NIH Guidelines and the Points To Consider Regarding NIH (ORDA) Review and Approval of Certain Categories of Human Gene Transfer Experiments That Qualify for the Accelerated Review Process

Dr. Robertson Parkman, Chair of the Working Group on Accelerated Review Protocols, will present an overview of the proposed amendments to the NIH Guidelines and the Points to Consider. The proposed amendments will: (1) Establish an accelerated review process for certain categories of human gene transfer experiments, (2) allow the National Institutes of Health (Office of Recombinant DNA Activities) to assign the appropriate review category to all human gene transfer proposals that are submitted in compliance with the NIH Guidelines, and (3) allow the National Institutes of Health (Office of Recombinant DNA Activities) to approve those categories of human gene transfer experiments that qualify for the accelerated review process in consultation with one or more Recombinant DNA Advisory Committee members, as necessary. Those human gene transfer experiments approved by the National Institutes of Health (Office of Recombinant DNA Activities) through the accelerated review process will be provided in a report by the Chair of the Recombinant DNA Advisory Committee at the next scheduled committee meeting. Those human gene transfer experiments approved by National Institutes of Health (Office of Recombinant DNA Activities) will be included in the list of approved experiments and will be available from the Office of Recombinant DNA Activities, Building 31, room 4B11, Bethesda, Maryland 20892.

XVI. Presentation on Informed Consent Issues

Dr. Gary Ellis, Director, Office for Protection from Research Risks, National Institutes of Health, Bethesda, Maryland, will address informed consent issues. Dr. Doris Zallen, Chair of the Working Group on Informed Consent, will lead a discussion concerning gene therapy protocols and informed consent.

XVII. Update on Cystic Fibrosis Protocol/Dr. Crystal

Dr. Ronald G. Crystal of the National Institutes of Health, Bethesda, Maryland, will present an update on his protocol entitled: A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592, June 11, 1980) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally, NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined not to be cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

Dated: November 2, 1993.

Daryl A. Chamblee,

Acting Deputy Director for Science Policy and Technology Transfer.

[FR Doc. 93–27572 Filed 11–8–93; 8:45 am]

BILLING CODE 4140–01–M

RECOMBINANT DNA ADVISORY COMMITTEE

Minutes of Meeting

December 2-3, 1993

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE
MINUTES OF MEETING[1]
December 2-3, 1993

The Recombinant DNA Advisory Committee (RAC) was convened for its fifty-sixth meeting at 9:00 a.m. on December 2, 1993, at the National Institutes of Health (NIH), Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy B. Walters (Chair) presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee Members:

Constance E. Brinckerhoff, Dartmouth Medical School
Ira H. Carmen, University of Illinois
Gary A. Chase, Johns Hopkins University
Patricia A. DeLeon, University of Delaware
Roy H. Doi, University of California, Davis
E. Peter Geiduschek, University of California, San Diego
Mariann Grossman, Hospital of the University of Pennsylvania
Robert Haselkorn, University of Chicago
Donald J. Krogstad, Tulane University School of Medicine
Brigid G. Leventhal, Johns Hopkins Hospital
Abbey S. Meyers, National Organization for Rare Disorders
A. Dusty Miller, Fred Hutchinson Cancer Research Center
Robertson Parkman, Childrens Hospital of Los Angeles
Leonard E. Post, Parke-Davis Pharmaceutical Division
Marian G. Secundy, Howard University College of Medicine
Brian R. Smith, Yale University School of Medicine
Stephen E. Straus, National Institutes of Health
LeRoy B. Walters, Kennedy Institute of Ethics, Georgetown University
Doris T. Zallen, VA Polytechnic Institute & State University

Executive Secretary:

Nelson A. Wivel, National Institutes of Health

A committee roster is attached (Attachment I).

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[1]The RAC is advisory to the National Institutes of Health (NIH), and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

National Institutes of Health Staff:

Leon Baltrucki, NHLBI
Bobbi Bennett, OD
Steve Brody, NHLBI
Diane Bronzert, NCI
Sarah Carr, OD
Jan Casadei, NCI
Shan Chu, NHLBI
Claire Danel, NHLBI
F. William Dommel, OD
Tony Eissa, NHLBI
Gary Ellis, OD
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Judy Fradkin, NIDDK
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Barry Goldspiel, NCI
Madelon Halula, NIAID
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Christine Ireland, OD
Susan Jenks, NCI
Sachiko Kajigaya, NHLBI
Stefan Karlsson, NINDS
Dai Katayose, NHLBI
Masako Kawase, NHLBI
Becky Lawson, OD
Johnson Liu, NHLBI
Catherine McKeon, NIDDK
Koichi Miyamura, NHLBI
Richard Morgan, NCHGR
Eric Oshiro, NINDS
Joan Porter, OD
Zvi Ram, NINDS
Melissa Rosenfeld, NHLBI
Nava Sarver, NIAID
Prem Seth, NHLBI
Kyoichi Shibuya, NCI
Thomas Shih, OD
Mario Sznol, NCI
Dennis Taub, NCI
John Villa, NINDS
Margo Warren, NINDS
Debra Wilson, OD
Haiping Wu, NHLBI
Neal Young, NHLBI

Others:

Paul Aebersold, Food and Drug Administration
Robert Anderson, Food and Drug Administration
W. French Anderson, University of Southern California
Briget Binko, Cell Genesys, Inc.
Andrew Braun, Harvard University
G'dali Braverman, ActUp
Joe Bruder, GenVec, Inc.
George Buffleben, Public
Gracia Buffleben, Public
Jeffrey Carey, Genetic Therapy, Inc.
Rachel Carle, Genzyme Corporation
Mike Casey, Genetic Therapy, Inc.
Fred Chang, University of Michigan Medical Center
Henry Chang, Shared Medical Research Foundation
Jan Chapell, Genetic Therapy, Inc.
Seng Cheng, Genzyme Corporation
Yawen Chiang, Genetic Therapy, Inc.
Larry Couture, Genzyme Corporation
Ronald Crystal, New York Hospital/Cornell Medical Center
Aileen Deist, Genetic Therapy, Inc.
Anne Driscoll, Fox, Bennett, and Turner
Eli Embley, F-D-C Reports, Inc.
Habib Fakhrai, San Diego Cancer Research Institute
Susan Falen, Genetic Therapy, Inc.
Jeffrey Fox, ASM News and Biotechnology
Joyce Frey, Food and Drug Administration
Ray Frizzell, University of Alabama, Birmingham
Morgan Gale, Hearings-on-the-Line
Alan Goldhammer, Biotechnology Industry Organization
Scott Graham, University of Iowa
Adam Grossman, ProTravel International
Lisa Grossman, ProTravel International
Robert Grossman, ProTravel International
Richard Haubrich, University of California, San Diego Treatment Center
Douglas Hickman, T. Rowe Price
David Holzman, BioWorld
Tom Horiagon, Chimerix Corporation
Edie Irvine, Genetic Therapy, Inc.
Burkhard Jansen, University of Vienna
Jack Jaugstetter, Genentech, Inc.
Rachel King, Genetic Therapy, Inc.
Toshi Kotani, Genetic Therapy, Inc.
John Kovach, Mayo Clinic
Imre Kovesdi, GenVec, Inc.

Steven Kradjian, Vical, Inc.
John Krauss, University of Michigan Medical Center
James Logan, University of Alabama, Birmingham
Donald Longenecker, Viagene, Inc.
Stephen Lupton, Targeted Genetics Corporation
Dan Maneval, Canji, Inc.
Tony Marcel, TMC Development
Keith March, Genetic Therapy, Inc.
Steve Marcus, Genetic Therapy, Inc.
Alan McClelland, Genetic Therapy, Inc.
Gerard McGarrity, Genetic Therapy, Inc.
Duncan McVey, GenVec, Inc.
Bruce Merchant, Viagene, Inc.
Andra Miller, Food and Drug Administration
Karen Millison, Genetic Therapy, Inc.
Gaye Morgenthaler, Murenove, Inc.
Gary Nabel, University of Michigan
Jeffrey Ostrove, Microbiological Associates, Inc.
Joseph Palca, National Public Radio
Hubert Pehamburger, University of Vienna
Michael Pensiero, Genetic Therapy, Inc.
Anne Petruska, The Blue Sheet
Stephen Pijar, University of Maryland
Paul Recer, Associated Press
Thomas Reynolds, Targeted Genetics Corporation
Rex Rhein, Biotechnology Newswatch
Joseph Rokovich, Somatix, Inc.
Alan Schreiber, Vical, Inc.
Friedrich Schuening, Fred Hutchinson Cancer Research Center
Richard Scotland, Genzyme Corporation
G. Terry Sharrer, Smithsonian Institution
Tomiko Shimada, Ambience Awareness International
Juliette Singh, Baxter Health Care
Alan Smith, Genzyme Corporation
Robert Sobol, San Diego Cancer Research Institute
Eric Sorscher, University of Alabama, Birmingham
Judith St. George, Genzyme Corporation
Marcus Stern, Copley News Service
Nevin Summers, Ingenex, Inc.
Jennifer Sutton, Association of American Medical Colleges
Paul Tolstoshev, Genetic Therapy, Inc.
Bruce Trapnell, Genetic Therapy, Inc.
Cynthia Utley, GenVec, Inc.
Christine VandePol, Rhone Poulenc Rorer, Inc.
Samuel Wadsworth, Genzyme Corporation
Trish Waitschies, MD Anderson Cancer Center

John Warner, Viagene, Inc.
Michael Welsh, University of Iowa
Katie Whartenby, Food and Drug Administration
Chet Whitley, University of Minnesota
Tom Wickham, GenVec, Inc.
M. Douglas Winship, Curative Technologies, Inc.
Janet Woodcock, Food and Drug Administration
Joseph Zabner, University of Iowa
Gregory Zaic, Prince Ventures

I. CALL TO ORDER

Dr. Walters (Chair) called the meeting to order and stated that notice of the meeting was published in the *Federal Register* on November 9, 1993 (58 FR 59612), as required by the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. He noted that a quorum was present and outlined the order in which speakers would be recognized. The primary and secondary reviewers will present their comments regarding the proposal, followed by responses from the principal investigators (PIs). The Chair will then recognize other committee members, *ad hoc* consultants, other NIH and Federal employees, the public who have submitted written statements prior to the meeting, followed by the public at large.

Dr. Walters stated that the number of human gene therapy protocols submitted for RAC review has rapidly increased over the last 3 years. He noted that 2 gene therapy trials were approved by the RAC in 1990, 3 trials in 1991, 10 trials in 1992, and 21 trials in the first 3 meetings of 1993. To date, the RAC has recommended approval of 36 human gene therapy trials to the NIH Director (does not include gene marking studies). (See Attachment II for complete protocol listing).

Dr. Walters stated that a favorable response was received to the RAC's recommendation regarding provision of medical care for subjects injured during the course of their participation in research. On August 6, 1993, Dr. Ruth Kirschstein, Acting NIH Director, responded to the RAC stating that she would forward the recommendations to Dr. Philip Lee, Assistant Secretary for Health, Department of Health and Human Services. In a letter dated October 8, 1993, Dr. Cliff Gaus (health care reform coordinator for Dr. Lee), indicated that the benefits package now envisioned for health care reform would probably cover services to subjects injured in clinical research. A brief discussion ensued about the interim policy regarding this issue.

Dr. Walters stated that the Office of Recombinant DNA Activities (ORDA) forwarded letters to two Institutional Review Boards (IRBs): (1) St. Jude's Children's Research Hospital to notify them of the RAC's recommendation to divide the Informed Consent document into two separate documents: a guardian consent form and a child's assent form (Dr. Kun's protocol). (2) University of California, San Diego (UCSD) to notify them of the RAC's requirement that prophylactic azidothymidine (AZT) administration be deleted from the protocol and that rabbit pyrogen testing not be mandatory (Dr. Wong-Staal's protocol). As a point of clarification, Dr. Parkman requested that the issue of AZT administration be verified.

II. MINUTES OF THE SEPTEMBER 9-10, 1993, RAC MEETING

Dr. Walters called on Dr. Carmen to review the minutes of the September 9-10, 1993, RAC meeting. Dr. Carmen summarized Dr. Hirano's written comments on the minutes and concurred that the minutes were an accurate reflection of the September meeting and recommended their approval. Minor changes were submitted by Drs. Miller and Zallen.

Committee Motion

The RAC approved a motion made by Dr. Carmen and seconded by Dr. Leventhal to accept the September 9-10 RAC minutes with the inclusion of minor changes by a vote of 15 in favor, 0 opposed, and no abstentions.

III. CHAIR REPORT ON MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS/DR. WALTERS

Dr. Walters stated that the RAC had approved minor modifications to the following RAC-approved human gene transfer protocols since the September 9-10, 1993, RAC meeting (See Attachment III for a complete list of minor modifications approved to date):

PROTOCOL	PRINCIPAL INVESTIGATOR	DATE APPROVED
9212-034	Ronald G. Crystal	October 8, 1992
9212-036	Michael J. Welsh	October 18, 1993
9209-030	Albert B. Deisseroth	November 3, 1993
9209-030	Albert B. Deisseroth	November 18, 1993
9212-034	Ronald G. Crystal	November 29, 1993
9206-019	Edward H. Oldfield	November 29, 1993

Dr. Walters noted that the only major concern in reviewing and approving these minor modifications was the addition of bronchial biopsies requested by Dr. Crystal. This procedure was not originally approved for this protocol; although other RAC-approved cystic fibrosis (CF) protocols include this procedure. After consultation with pulmonologists, Dr. Walters concluded that bronchial biopsy, as proposed for this protocol, poses less risk to patients than transbronchial biopsy.

IV. WORKING GROUP ON DATA MANAGEMENT - SEMI-ANNUAL DATA REPORT/DR. LEVENTHAL

Dr. Walters called on Dr. Leventhal to summarize the semi-annual data reports submitted by the PIs of NIH-approved human gene transfer protocols. Dr. Leventhal stated that some investigators failed to provide adequate responses to the questions regarding possible adverse effects and evidence of gene transfer. She recommended that the Working Group on Data Management should have an interim meeting to revise the reporting requirements.

A total of 150 patients have been accrued onto the 50 NIH Director-approved protocols to date. Of these 50 studies, 7 are closed, 20 have had no patient accrual to date, and 12 are pending approval by the Food and Drug Administration (FDA). Summarized below are the categories of human gene transfer protocols that have been approved by the

RAC to date (See Attachment IV for complete data management report):

	Therapy	Marking	Total (T + M)
RAC Approved	35	23	58
NIH Director Approved	28	22	50
Categories of RAC-Approved Protocols			
Cancer	26	4	30
Cystic Fibrosis	4	1	5
SCID/ADA	1	0	1
Acute Hepatic Failure	0	1	1
Familial Hypercholesterolemia	1	0	1
Gaucher Disease	2	0	2
Bone Marrow Marking/Cancer	0	15	15
HIV(+)	2	2	4

The next review of human gene transfer data will be presented at the June 9-10, 1994, RAC meeting. Dr. Leventhal noted several issues that should be addressed by the working group prior to the June 1994 data reporting period. PIs should not be required to submit semi-annual data reports as a follow-up to protocols that have been closed for >1 year. She stated concern about the issue of under- and over-accrual of patients into NIH-approved protocols. Another issue is the lack of reporting unexpected toxicity and adverse reactions. Any such observation should be reported to the RAC, particularly in light of recent severe adverse reactions encountered in the fialuridine, or FIAU, hepatitis drug trial at NIH. Dr. Janet Woodcock of the FDA stated that the FDA requires that any unexpected adverse reaction must be reported immediately. All events, expected or unexpected, are reported annually and at the close of each study. Dr. Leventhal suggested that PIs submit copies of FDA adverse reaction reports to the RAC.

Discussion ensued regarding recent press reports and public expectations about human gene therapy and the current state of art of this new biotechnology. The majority of the human gene transfer trials that have been approved are Phase I/II studies; therefore, the trials are not designed to assess efficacy. These Phase I/II studies should be evaluated on the basis of whether proposed scientific objectives have been obtained.

V. UPDATE ON THE OLDFIELD PROTOCOL ENTITLED: *GENE THERAPY FOR THE TREATMENT OF BRAIN TUMORS USING INTRA-TUMORAL TRANSDUCTION WITH THE THYMIDINE KINASE GENE AND INTRAVENOUS GANCICLOVIR* (#9206-019)/DR. RAM

Dr. Walters called on Dr. Zvi Ram of the NIH, Bethesda, Maryland, to provide an update on this human gene transfer protocol. Dr. Ram stated that as previously reported

to the RAC, patient #1 developed a secondary brain tumor following injection of vector producing cells (VPC) and subsequent administration of ganciclovir. Some RAC members were initially concerned that this secondary tumor may have been related to the gene transfer procedure. The first brain tumor was diagnosed as an anaplastic renal cell metastasis. When the patient developed a glioblastoma at a site distant from the original tumor subsequent to gene transfer, extensive analyses were performed. Molecular analysis demonstrated no evidence of the retroviral vector or the VPC in the second tumor (glioblastoma). Patient #1 demonstrated progressive disease that led to death. Extensive post-mortem studies were conducted, and subsequent analyses demonstrated that the first brain tumor was misdiagnosed. The original brain mass was not a renal cell carcinoma metastasis, but a primary glioblastoma. Patient #1 had two primary tumors, a renal cell carcinoma and a glioblastoma. Subsequent progression of the glioblastoma resulted in the patient's death. Dr. Leventhal noted that she reviewed the data submitted by the investigators and concurs with this assessment.

VI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *INTRATHECAL GENE THERAPY FOR THE TREATMENT OF LEPTOMENINGEAL CARCINOMATOSIS*/DRS. OLDFIELD AND RAM

Review--Dr. Smith

Dr. Walters called on Dr. Smith to present his primary review of the protocol submitted by Drs. Edward H. Oldfield and Zvi Ram of NIH, Bethesda, Maryland. Dr. Smith stated that the PIs propose to use the herpes simplex virus thymidine kinase (HSV-tk) gene/ganciclovir (GCV) strategy that has been previously approved by the RAC for other malignancies. Leptomeningeal carcinomatosis is a fatal disease; therefore, a probable candidate for gene transfer research. The proposed vector, G1TK1SvNa, is similar to the previously approved construct. The packaging cell line, PA317, has been approved previously. A total of 20 patients will be entered into the study. Patients will receive intraventricular and/or intrathecal injections of VPC. The end points of the study are to: (1) assess toxicity (i.e., cerebral spinal fluid (CSF) meningitis symptoms and/or obstruction induced by placing up to 8×10^9 VPC cells in 120 ml of CSF, and any adverse reactions such as the asymptomatic gliosis encountered in the previous trial; (2) determine the kinetics and distribution of the VPC following injection into the CSF and CSF vector titer; and (3) determine clinical efficacy as assessed by standard tumor cell markers and magnetic resonance imaging (MRI).

Dr. Smith expressed concern about the asymptomatic gliosis that was encountered in the investigators' previous brain tumor protocol. He asked the PI to summarize the most recent data regarding this observation and any correlations with the proposed study. The number of VPC proposed for this study is greater than the number of VPC used for the rhesus monkey studies. This larger number of cells may increase the risk of CSF blockade and other meningeal complications. The β-galactosidase reporter gene was used successfully as a marker to assess gene transfer in the preclinical studies; therefore, this reporter gene should yield valuable information about HSV-tk transduction of tumor

cells in the CSF in the human trial. He asked the investigators whether there is any data demonstrating efficacy of HSV-tk gene transfer and GCV administration on breast and lung tumor cells. He concluded that most of his initial concerns were adequately addressed, and recommended approval of the protocol.

Review--Dr. Brinckerhoff

Dr. Brinckerhoff said that the proposed study is clearly described and thoroughly documented. This protocol is a direct extension of previously approved HSV-tk/GCV brain tumor studies. Clinical efficacy has been demonstrated in the rat model. Of the 8 patients treated on Dr. Oldfield's glioblastoma protocol, 5 have demonstrated an antitumor response. Injection of the VPC and subsequent GCV administration has been well tolerated in all patients; this observation lends strong support for the present proposal. Dr. Brinckerhoff posed the following questions. Does the transduction of dividing cells in the subarachnoid space include macrophages and epithelial cells as well as tumor cells? Are the VPC capable of passing out of the central nervous system, circulating in the blood, and eliciting an immune response? Are there possible cryptic transcription start sites in the vector construct? She concluded that the proposed study could potentially prolong survival of these patients by a relatively non-invasive means and may serve as a paradigm for future gene therapy protocols; therefore, the RAC should recommend approval.

Other Comments

Dr. Leventhal asked whether the proposed number of patients is sufficient to evaluate efficacy. Dr. Ram responded that this study is not designed to evaluate efficacy. The total number of patients will be divided into 4 dose-escalation groups.

Investigator Response--Dr. Ram

Dr. Ram summarized the results obtained from Dr. Oldfield's previously approved glioblastoma protocol which is analogous to this study. Twelve patients (10 surgically inaccessible and 2 surgically accessible) have received HSV-tk/ganciclovir administration. In the surgically accessible group, patients' tumors were resected 1 week following VPC injection and subsequently analyzed for *in vivo* evidence of gene transfer. The objectives of the study (i.e., safety, antitumor response, and *in vivo* gene transfer) were achieved and there has been no evidence of immediate or delayed toxicity. Although the study was not designed to be curative, significant antitumor responses have been demonstrated. A subgroup of patients demonstrated no evidence of antitumor activity, and their tumors rapidly progressed for undetermined reasons. Preliminary *in situ* hybridization assays of resected tumors indicate successful gene transfer into tumor cells at the VPC injection site.

Dr. Miller remarked that these glioblastoma patients have a limited life expectancy which precludes any observation of long-term safety. Dr. Ram emphasized that the proposed leptomeningeal carcinomatosis study will yield valuable scientific information

for a uniformly fatal disease. CSF circulates around the brain and spinal cord and comes in contact with every cell of the leptomeninges. Injection of VPC into this space will yield important information about the pharmacodynamics of GCV and distribution of the VPC and vector. Such data will be valuable for future gene therapy applications.

Dr. Haselkorn asked if tumor markers exist that would allow the investigators to distinguish between responding and non-responding tumors. Dr. Ram answered that although a few markers exist for glioblastoma, there are no known markers for leptomeningeal carcinomatosis. Dr. Doi asked if experiments had been performed to look for the presence of the HSV-tk gene in those patients who exhibited rapidly growing tumors. Dr. Ram responded that the biology of the tumor often changes after several resections. Dr. Miller stated that evidence of antitumor response justifies the expansion of this treatment to other diseases.

To follow up on Dr. Doi's question, Ms. Grossman questioned whether the HSV-tk gene sequences were detected in the non-responding tumor group. Dr. Ram answered that *in situ* hybridization assays were performed only on resected tumor specimens obtained from the surgically accessible group. Such analysis may be inconclusive since the specimens were obtained 7 days after GCV treatment which eliminates the HSV-tk transduced cells.

Dr. Parkman asked how the dynamics and distribution of VPC will be assessed. Will tumors block the ventricular system and the subarachnoid space? Dr. Ram replied that one of the exclusion criterion of the proposed study is evidence of such a blockage. CSF samples will be obtained from the ventricular system and from the lumbar spinal cord to monitor the dynamics and distribution of the vector and VPC. Dr. Ram noted that *in vivo* monkey experiments demonstrated even distribution of the vector and VPC between the cervical and lumbar spinal cord.

Dr. Miller asked why post-mortem studies were not performed on most of the glioblastoma patients. Dr. Ram explained that it is often difficult to obtain permission from relatives, etc., to obtain post-mortem brain tissue. Dr. Leventhal acknowledged that such permission is often difficult to obtain.

Committee Motion

A motion was made by Dr. Smith and seconded by Dr. Secundy to approve the protocol. The motion passed by a vote of 13 in favor, 1 opposed, and 1 abstention.

VII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *RETROVIRAL MEDIATED GENE TRANSFER OF THE FANCONI ANEMIA COMPLEMENTATION GROUP C GENE TO HEMATOPOIETIC PROGENITORS OF GROUP C PATIENTS*/DRS. LIU AND YOUNG

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Drs. Johnson M. Liu and Neal S. Young of NIH, Bethesda, Maryland. Dr. Parkman stated that Fanconi anemia is an autosomal recessive genetic disease in which the affected individuals have an inability to repair spontaneous or induced DNA breaks. As a consequence of their decreased capacity to repair DNA breaks, patients develop aplastic anemia, have an increased likelihood of cancer development, and may have anatomic abnormalities involving the skeleton and kidney. Four complementation groups of Fanconi anemia have been defined. The gene responsible for the defect, Fanconi anemia complementation C (FACC) has been identified and cloned. The protein product of FACC is 63 kd; however, the function of this protein is unknown. The investigators have demonstrated that *in vitro* transduction of the FACC gene into patients' cells (B lymphoblast cell lines and target CD34(+) cells) results in the normalization of the sensitivity of the transduced cells to mitomycin C treatment and a reduction in the number of induced chromosomal breaks. The investigators have demonstrated in this preclinical model that transduction of abnormal cells with the FACC gene can result in the physiological normalization of these cells. The proposed study is similar to the Gaucher's disease protocols previously approved by the RAC in that CD34(+) cells will be isolated from peripheral blood following granulocyte colony stimulating factor (G-CSF) stimulation. The use of peripheral blood cells eliminates the possible risk associated with the use of general anesthesia for bone marrow harvesting. Questions still exist about the relative quality and frequency of obtaining true pluripotent hematopoietic stem cells from peripheral blood versus bone marrow cells. Considering the risks of general anesthesia, the present approach is appropriate for the proposed study. CD34(+) cells will be isolated using the CellPro® monoclonal antibody column and transduced *in vitro* with the retroviral vector in the presence of the growth factors, interleukin-3 (IL-3), interleukin-6 (IL-6), and G-CSF. The cells will be reinfused into patients following transduction. Patients will receive a maximum of 4 infusions over a period of 12 months, at a frequency of ≥ every 2 months. Peripheral blood and bone marrow cells will be analyzed for the presence of the transduced FACC gene and its functional effect of conferring resistance to mitomycin C. This protocol is a logical extension of the investigators' previous work on Gaucher's disease and the Autologous Bone Marrow Transplant Program at NIH. If a significant *in vivo* selective growth advantage is demonstrated for the transduced stem cells and their progeny, this study may result in a potential clinical benefit. The investigators have adequately responded to most of the issues raised in the primary review.

Review--Dr. Smith

Dr. Smith stated that although the investigators partially responded to some of the questions posed in his written comments, there are several issues that require further discussion. One major concern is the use of growth factors both in patients and during the transduction procedure. CD34(+) cells will be harvested following mobilization with G-CSF. In patients with non-Fanconi's myelodysplastic syndromes, growth-factor administration can result in elevated blast counts, presenting the theoretical risk of accelerating progression to acute leukemia. Similarly, *ex vivo* treatment of the harvested progenitor cells with a cocktail of experimental growth factors (IL-3, G-CSF, and IL-6)

carries a similar risk of increasing the susceptibility of an abnormal stem cell becoming leukemic or furthering the growth potential of an already extant leukemic cell clone. This theoretical risk is not adequately described in the protocol. The investigators have not stated clearly that the proposed treatment only attempts to correct the hematopoietic cells, not other cells that are affected by this disease. The investigators have only provided short-term tissue culture data. Dr. Smith posed the following questions. What are the long-term effects of inserting the FACC gene under constitutive expression conditions that alter normal cellular function? Will the transduced cells be selected in G418? Can adequate numbers of CD34(+) cells be harvested from these aplastic patients? There is little information available about how the gene defect actually results in disease. Fanconi anemia is an appropriate candidate for gene transfer experiments and the investigators are well qualified to conduct such experiments; however, these issues must be addressed before the RAC makes a recommendation regarding approval.

Review--Dr. Carmen

Dr. Carmen stated that since the RAC has never reviewed any human gene transfer proposal for the treatment of the genetic disease Fanconi anemia, the RAC should provide a careful review of this novel approach. The investigators have not provided data in an appropriate animal model, citing the fact that the exact biochemical function of the FACC gene is unknown. The protocol currently states that the PIs are "currently investigating the effect of long-term expression of the gene in a murine transplantation model." Ordinarily, recommending approval of a protocol that does not include a preclinical animal model is unusual, especially since patients will be ≤ 18 years of age. However, the investigators have provided adequate human *ex vivo* data to support the proposed study. Lymphoblast cell lines and CD34(+) cells isolated from Fanconi anemia patients were restored to their normal sensitivity to mitomycin C treatment following transduction with the FACC retroviral vector. He recommended modifications to the Informed Consent document that would make the language more understandable to laypersons and recommended approval of the protocol contingent on the acceptance of the modified Informed Consent document language.

Other Comments

Dr. Post expressed concern about the possible overproduction of the FACC protein, which has an unknown function in hematopoietic cells. He asked the investigators to provide additional information about the murine transplantation experiment that was conducted to determine the constitutive expression of the FACC gene.

Investigator Response--Dr. Liu

In response to Dr. Smith's concern about the use of growth factors in Fanconi anemia patients, Dr. Liu said that G-CSF has been used previously to treat several Fanconi anemia patients with severe pancytopenia for up to 1 year. One patient demonstrated a spontaneous clonal karyotypic abnormality prior to G-CSF treatment. To date, there is no evidence that treatment of Fanconi anemia patients with G-CSF poses the risk of

conversion from preleukemic to a leukemic state. Dr. Liu acknowledged that there is a theoretical concern about leukemic conversion which led the PIs to limit the administration of G-CSF to a 1 week period. Patients will be placed under close observation for this potential risk. Drs. Smith and Doi expressed concern that patients will be included who have clonal karyotypic abnormalities of their blood cells. Dr. Liu responded that retrospective studies of Fanconi anemia patients suggest that there is no association of clonal karyotypic abnormalities with the onset of leukemia. Fanconi anemia is a very rare genetic disease, which places severe limitations on the eligibility criteria. In response to Dr. Smith's question regarding the use of growth factors during the *ex vivo* transduction procedure, Dr. Liu stated that these procedures are designed to stimulate hematopoietic progenitor cells to divide in order to enhance transduction efficiency. There is very limited experience to indicate whether these factors are necessary for transduction of cells from Fanconi anemia patients. Dr. Smith was concerned that growth factors such as IL-6, IL-3, and G-CSF could result in the outgrowth of preleukemic cells during the transduction procedure. Dr. Liu explained that these growth factors have been used previously to treat patients who either are preleukemic or leukemic. There is no data indicating that these patients are at high risk of accelerating leukemia development. Dr. Post remarked that Dr. Cynthia Dunbar's semi-annual data report form (RAC protocol #9206-025) indicated that recent data suggests the possibility that G-CSF may favor the growth of leukemic versus normal progenitors during the *ex vivo* culture. Dr. Post agreed with Dr. Smith's concern about the use of growth factors during the transduction of cells from Fanconi anemia patients. Dr. Young, co-PI on the proposed study, responded that the *in vitro* data demonstrating leukemia cell proliferations by G-CSF or GM-CSF (granulocyte-macrophage colony stimulating factor) involved cells obtained from patients with acute or chronic myelogenous leukemia. This proposal involves Fanconi anemia patients, not myelogenous leukemia patients. Dr. Young stated that it is unreasonable to exclude the use of growth factors based on *in vitro* observations. Dr. Smith suggested that the investigators should contact Dr. Dunbar and request additional data regarding her observations.

In response to Dr. Post's question about murine transplantation experiments, Dr. Liu stated that these studies are ongoing. Bone marrow was obtained from C57/Bl6 mice and transduced with the FACC vector. These transduced cells were then reinfused into recipient W/W^v mice. Preliminary data indicate successful engraftment and transduction of the FACC gene into the stem cells of recipient mice. No abnormalities have been observed by necropsy of one recipient. Data are currently unavailable with regard to *in vivo* expression or transduction efficiency of the FACC gene. Ms. Grossman expressed her reservations about the lack of data derived from a relevant animal model. Dr. Miller remarked that the murine model will be useful to address many of the safety issues that have been posed by the RAC. If the transduced FACC gene is adequately expressed in mice, concerns about untoward effects of the FACC gene on normal cells can be addressed. Dr. Liu said that the suggested *in vivo* studies are in progress.

Committee Motion #1

A motion was made by Ms. Grossman and seconded by Dr. Secundy to defer the protocol based on the lack of *in vivo* data. Dr. Walters invited discussion on this motion. Dr. Post said that although the ongoing murine studies will address several safety issues, the data are incomplete. Dr. Post urged the investigators to further characterize the ongoing experiments in order to demonstrate the safety of FACC gene expression in mice.

Ms. Meyers commented that Fanconi anemia is a very rare disease, and little is known as compared to other more common genetic disorders. She expressed concern that there may never be an adequate amount of data to address all the RAC's concerns. She suggested that a more appropriate motion would be to approve the protocol contingent upon receipt of data derived from ongoing animal studies.

Dr. Parkman expressed concern that approval of this protocol, which involves gene transfer for the treatment of a genetic disease that has not been previously reviewed by the RAC, would set a precedent for other novel protocols. Although the investigators have submitted efficacy data based on an *in vitro* model, little is known about the safety of long-term expression of the FACC gene in hematopoietic stem cells. Dr. Parkman noted that similar murine experiments were not required for RAC approval of other protocols, such as the adenosine deaminase (ADA)-deficiency protocol which also involved stem cell transduction.

Dr. Smith stated he is in favor of approving the protocol contingent on the submission of the additional data noted by Dr. Dunbar and review and approval of data obtained from the ongoing murine experiments. Dr. Post stated his view that the protocol should be deferred until the investigators return to the full RAC with the additional safety data. Dr. Miller stated that the protocol should be approved contingent on the submission of data from ongoing experiments; and recommended a 4 month follow-up for toxicity experiments would be adequate. Dr. Liu responded that 8 mice have been followed for a period of 3 months.

Dr. Walters asked Dr. Miller if he would like to offer a substitute motion for the original deferral motion made by Ms. Grossman.

Committee Motion #2

A substitute motion was made by Dr. Miller and seconded by Dr. DeLeon to conditionally approve this protocol contingent upon the receipt of the murine safety data to be reviewed by a subcommittee that includes the primary reviewers. Dr. Miller said that this motion will not delay initiation of the protocol. The RAC voted to accept Dr. Miller's substitute motion by a vote of 9 in favor, 6 opposed, and no abstentions.

Dr. Leventhal expressed her reservation about allowing a subcommittee to review the requested data. The RAC meets every 3 months; therefore, there is no reason that the investigators cannot present data derived from the ongoing studies at the next RAC meeting. Dr. Smith agreed that full RAC review would afford a more detailed

examination of the data. Ms. Grossman stated her preference to defer approval of the protocol until the next meeting. Dr. Chase stated that the scientific review of these data could be adequately addressed by a subcommittee. Dr. Secundy disagreed with Dr. Chase's comments noting that public members offer valuable contributions to such reviews.

Dr. Doi suggested that the RAC should specify their recommendations to the investigators. Dr. Miller said that the mice involved in the ongoing toxicity studies should be observed for a period of 4 months based on the fact that this length of time is required to evaluate engraftment of transduced stem cells in mice. Dr. Smith said that in addition to the animal data, the RAC should have access to Dr. Dunbar's data demonstrating the effect of growth factors on the *ex vivo* transduction of stem cells. Dr. Liu asked if he would be required to submit the additional data 8 weeks prior to the next RAC meeting and whether resubmission of the entire protocol would be mandatory.

Dr. Secundy suggested that the Informed Consent document should be amended to include bone marrow examination as a condition for continuation in the protocol. Ms. Grossman, Drs. Parkman, Leventhal, and Zallen all stated that bone marrow examination with each reinfusion cycle should be an inclusion criterion in order to obtain scientifically meaningful data. Dr. Liu agreed to include this requirement in the protocol and in the Informed Consent document.

Dr. Walters called for a vote on the motion to conditionally approve the protocol contingent on the review of data derived from ongoing safety experiments by a subcommittee of the RAC. The motion was defeated by a vote of 5 in favor, 10 opposed, and 1 abstention. Dr. Parkman suggested that a new motion should waive the 8 week deadline for submission and allow the investigators to resubmit the protocol up to 4 weeks prior to the next RAC meeting.

Committee Motion #3

A motion was made by Dr. Miller and seconded by Dr. Secundy to defer approval of the protocol until the investigators return to the full RAC with the following: (1) murine data demonstrating *in vivo* expression of the FACC gene and safety data accumulated over a period of ≥ 4 months demonstrating that the FACC-transduced cells do not result in any untoward effects; (2) data as cited in Dr. Cynthia Dunbar's semi-annual data report (RAC protocol # 9206-025) regarding the possibility that "stem cell factor could favor the growth of leukemic versus normal progenitors during *ex vivo* culture periods;" and (3) revisions to both the protocol and Informed Consent document to modify the eligibility criteria regarding the necessity for bone marrow examination following each reinfusion.

The consensus of the RAC was that the investigators are not required to submit this additional data until 4 weeks prior to the RAC meeting at which time the information will be reviewed. Submission of previously reviewed information is not required. The motion to defer approval of the protocol pending full RAC review of additional data

passed by a vote of 14 in favor, 0 opposed, and 3 abstentions.

VIII. CONTINUATION OF DISCUSSION REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I CLINICAL TRIAL TO EVALUATE THE SAFETY AND EFFECTS IN HIV-1 INFECTED HUMANS OF AUTOLOGOUS LYMPHOCYTES TRANSDUCED WITH A RIBOZYME THAT CLEAVES HIV-1 RNA*/DR. WONG-STAAL (SEE PREVIOUS DISCUSSION UNDER CALL TO ORDER)

Dr. Parkman noted that the approval of Dr. Wong-Staal's human gene transfer protocol was contingent on the University of California, San Diego IRB eliminating the requirement for mandatory AZT administration. The transcripts of the September 9-10 meeting were reviewed, and this contingency was verified. ORDA noted that a letter of response has not yet been received from Dr. Wong-Staal's IRB.

IX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *INJECTION OF COLON CARCINOMA PATIENTS WITH AUTOLOGOUS IRRADIATED TUMOR CELLS AND FIBROBLASTS GENETICALLY MODIFIED TO SECRETE INTERLEUKIN-2*/DRS. SOBOL AND ROYSTON

Review--Dr. Miller

Dr. Walters called on Dr. Miller to present his primary review of the protocol submitted by Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California.

Dr. Miller explained that this Phase I study involves the injection of a mixture of autologous irradiated tumor cells and autologous irradiated IL-2-producing fibroblasts to stimulate antitumor immune responses in colon carcinoma patients. The fibroblasts will be engineered to produce IL-2 by using a replication-defective retroviral vector similar to other vectors that have been approved previously by the RAC. The proposal is well designed and addresses most issues related to the use of recombinant DNA in humans. The investigators have submitted preclinical data derived from the CT26 BALB/c colorectal carcinoma murine model.

Dr. Miller stated that the data does not entirely support the protocol, especially regarding the generation of systemic antitumor immunity. Although immunity was observed at a concentration of 100 units of IL-2/24 hours, antitumor immunity was not demonstrated at doses up to 1,700 units of IL-2/24 hours. The animal model is irrelevant because the murine studies employed non-irradiated IL-2-secreting fibroblasts. The human study involves irradiated IL-2-secreting fibroblasts. Have experiments been conducted with animals that have established tumors? Why is there low viability when the IL-2 secreting fibroblasts are irradiated at 3,000 rads? He stated that if these concerns are adequately addressed by the investigators, the protocol should be approved on the basis that generation of antitumor immune responses may hold promise for the treatment of human cancers, and the use of irradiated cells presents minimal risk to the

patients.

Review--Dr. Straus

Dr. Straus agreed with Dr. Miller's assessment about the minimal risk associated with the proposed study. Dr. Straus noted several initial concerns about the protocol, some of which were partially addressed by the PI's written responses. Dr. Straus expressed concern that the proposed vector is not identical to the previously approved vector, and safety data on the retrovirus lots has not been submitted. The protocol states that the fibroblasts and tumor cells will be lethally irradiated, yet data demonstrating the level of irradiation required for complete cell killing has not been provided. The investigators have not specified the exact treatment schedule or the number of patients they propose to enroll on the study. *In vivo* evidence of an antitumor response has not been demonstrated in animals with established disease. The Informed Consent document is exceedingly brief and many items, including the number and amount of blood drawings and possible side effects, have been omitted. Dr. Straus concluded that the protocol in its present form should not be approved.

Review--Dr. Zallen

Dr. Zallen stated that the nontechnical abstract is unacceptable because the language is not comprehensible to the laypersons. She stated that her concerns focus on 3 areas: (1) inadequate animal model studies, (2) the capability of the investigators to conduct the proposed experiment, and (3) the Informed Consent document. She noted that her initial concern about the use of the term "immunization" in the Informed Consent document has been corrected in the revised document. The *Risks and Discomforts Section* of the Informed Consent document does not adequately describe the possible allergic reactions and risks associated with replication-competent retroviruses (RCR). The statement in the Informed Consent document about financial responsibility of research costs to the patients is unacceptable. This document should clearly state that neither the patients nor their insurance companies are responsible for any of the direct costs arising from their participation in this study. In regard to preclinical data, the investigators should clarify the results obtained in the animal model, e.g., why are systemic antitumor immune responses only obtained from injection of tumor cells in the opposite flank? Are there any data that demonstrates immune cell activation in an *in vitro* system? Do the investigators possess adequate expertise to reproduce the number of viable tumor cells required for this study? The RAC should not approve this experiment based on the incomplete nature of the preclinical data, the unsatisfactory Informed Consent document, and the high risk/benefit ratio to these patients.

Other Comments

Dr. Haselkorn asked the investigators to summarize the information that was gained from the patient that was approved to receive gene transfer on a compassionate plea basis. Ms. Meyers suggested the inclusion of a statement in the Informed Consent document regarding patient confidentiality. Dr. Leventhal inquired about the process for

obtaining tumor specimens from patients whose primary tumor has already metastasized.

Investigator Response--Dr. Sobol

Dr. Sobol agreed to incorporate Ms. Meyers' suggestion about patient confidentiality into the Informed Consent document. In response to Dr. Leventhal's question about access to tumor specimens, Dr. Sobol stated that tumor cells will be obtained at the time of colon resection, even if the patient has metastatic disease. Those tumor specimens will be cryopreserved. Dr. Leventhal said that the Informed Consent document should be revised to include a statement that clearly explains that patients will have their primary tumors cryopreserved at the time of resection. Responding to questions about the *in vivo* data, Dr. Sobol said that 3 separate animal studies were performed. One experiment demonstrated an antitumor response effected by IL-2 transduced fibroblasts and noted that this response was not T cell-mediated. The second experiment demonstrated an antitumor response associated with a low level of IL-2 production; whereas, no antitumor response was observed at high levels of IL-2 production. A third experiment was conducted in which animals were first challenged with a live tumor transplant. Dr. Parkman explained that the animal studies are inconclusive regarding the optimum concentration of IL-2 for the proposed human study. Dr. Chase stated that although the protocol does not present any significant risk, there is no significant scientific conclusion derived from the preclinical studies that can be translated to the human study. Dr. Parkman noted that the investigators propose to treat colon cancer with the IL-2-producing cells, an aspect different from other previously approved protocols. Dr. Post added that animal experiments often are of limited value for human studies; therefore, the positive antitumor responses obtained from these studies should be considered.

Dr. Sobol explained that if no toxicity is observed, 9 patients will be treated (3 patients in each of the 3 dose groups). If toxicity is observed, a larger number of patients will be required to define a safe dose. Dr. Straus suggested that accrual should be limited to a maximum of 12 patients. Dr. Sobol agreed to accept this upper limit of patient accrual. Dr. Sobol stated that a volume of between 100 and 200 milliliters (ml) of blood will be drawn at each immunization (i.e., a total of 400 to 800 ml over a two month period). Dr. Straus remarked that this volume of blood is higher than the allowable research limit. Dr. Sobol agreed to use the lower volume, 100 ml, of blood to be drawn at each immunization. Dr. Sobol agreed to revise the Informed Consent document and nontechnical abstract as suggested by the RAC. Regarding the lethal irradiation of cells, Dr. Sobol said that additional experiments are ongoing to determine the lethal dose of radiation.

Committee Motion

A motion was made by Dr. Parkman and seconded by Dr. Post to approve the protocol. RAC approval of the protocol is contingent on the review and approval of the following by the primary reviewers: (1) a revised Informed Consent document (incorporating the changes suggested by RAC members), (2) patient accrual will be limited to $\leq$ 12 patients, (3) data demonstrating lethal irradiation of tumor cells and fibroblasts, and (4) a revised

patient eligibility criterion that limits the study to those patients who undergo treatment for their primary tumor (i.e., available tumor cryopreserved) at Sharp Memorial Hospital, San Diego, California. The motion to approve the protocol passed by a vote of 14 in favor, 0 opposed, and 2 abstentions.

X. **ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *INJECTION OF GLIOBLASTOMA PATIENTS WITH TUMOR CELLS GENETICALLY MODIFIED TO SECRETE INTERLEUKIN-2 (IL-2): A PHASE I STUDY*/DRS. SOBOL AND ROYSTON**

Review--Dr. Krogstad

Dr. Walters called on Dr. Krogstad to present his primary review of the protocol submitted by Drs. Robert E. Sobol and Ivor Royston of the San Diego Regional Cancer Center, San Diego, California. Dr. Krogstad said that this Phase I study involves glioblastoma patients. The objectives of this study are to: (1) to evaluate the safety of subcutaneous immunization with irradiated autologous or allogeneic HLA-A2 (human leukocyte antigen-A2) positive glioblastoma cells modified to secret IL-2, (2) evaluate the efficacy of these immunizations on tumor growth, (3) induce cellular or humoral responses by this process, and (4) compare responses induced by autologous versus allogeneic tumor cells. The rationale for this study is based on published data demonstrating that the HLA-A2 locus is a dominant haplotype for tumor antigen presentation. The investigators propose that peripheral immunization with IL-2 transduced allogeneic glioblastoma cells will induce immune responses that will cross the blood-brain barrier from the systemic circulation into the brain. The investigators cite a study by Mahaley, et. al., that suggests the prolonged survival of patients following such immunizations. However, this study is difficult to interpret because these patients underwent other forms of treatment simultaneously.

Dr. Krogstad explained that the investigators propose to use the retrovirus vector, G1NaCvI2.23 (Genetic Therapy, Inc), with a human IL-2 cDNA insert. This construct has been previously approved by the RAC for other human gene transfer studies. He expressed concern that allocating patients to 2 arms of the study may complicate interpretation of the data. He questioned whether the state of the art technologies (e.g., MRI and CT (computerized tomography)) will accurately assess questions of treatment, toxicity, and efficacy. Since the basis for this proposed study is data derived from a single patient study, issues such as toxicity and efficacy may not be accurately addressed. However, useful information may be gained about the immunologic effects of the proposed experiment.

Review--Dr. Chase

Dr. Chase agreed that useful information may be obtained from the immunological studies; however, this proposal is based on dubious neuroradiology of a single patient who received other forms of treatment (e.g., chemotherapy and radiotherapy) immediately prior to and during the course of the gene transfer study. Neither scientific

or medical information may be obtained from treating additional patients on a protocol that is based on non-definitive data. He recommended disapproval of this protocol.

Review--Ms. Meyers

Ms. Meyers said that many of her initial concerns about the Informed Consent document have been addressed by the investigators. However, there are several remaining issues that should be addressed by the PI. Why are the investigators proposing to use cryopreserved, allogeneic cells obtained from the deceased patient? She cited the California court decision (John Moore versus the University of California) in which the court ruled that any commercial preparation derived from a patient's cells is the property of that patient, and the manufacturer must obtain permission for the use of such cells and provide compensation. Has written permission been obtained for the use of these cells prior to the patient's death? Although a minimum expected survival (3 months) has been defined, a maximum expected survival (i.e., 1 year) should be included. The Informed Consent document should be revised to include a statement about patient confidentiality and protection from the media. She expressed her displeasure about the inaccurate press report by Reuters Information Services, Inc., which suggests that the single patient trial was efficacious.

Other Comments

As a follow-up to Ms. Meyers comments and regarding the inaccuracy of the Reuters press report about the single patient trial, Dr. Haselkorn asked the investigators about two quotations: (1) "the patient lived several months longer than the average life expectancy of a person suffering with the particular form of incurable brain cancer," and (2) "the fact that we were able to demonstrate an immune response against the tumor suggests additional evaluation of patients is warranted." Dr. Leventhal expressed her objection to the statements made in the Reuters news report. The patient treated on the single patient trial received Decadron, Tamoxifen, and other forms of antitumor therapy. It is impossible to draw any scientific conclusions about the effects of the gene transfer. Dr. Leventhal disagreed with the investigators' interpretation of the immunological data obtained from the single patient. The preliminary data does not support approval of this proposal.

Dr. Walters called on Dr. Parkman to interpret the immunological data obtained from the previous patient. Dr. Parkman stated that the investigators used an *in vitro* system to evaluate the cytolytic capacity of peripheral blood lymphocytes. The data derived from these *in vitro* experiments demonstrates a slight increase in cytolytic activity in response to this treatment.

Investigator Response--Dr. Sobol

In response to Ms. Meyers' concern that an autopsy was not obtained on the single patient who died while on the previous trial, Dr. Sobol explained that the family denied the request for autopsy. However, *in vitro* experiments were performed with cells

obtained from this patient. Although the data suggest an increase in cytotoxic T lymphocyte (CTL) activity following tumor cell injections, the responses were variable. The reasons for this variation are unclear. Dr. Smith inquired about the numbers of times that patient's pretreatment level was measured. Dr. Sobol responded that this assay had been performed twice. Drs. Smith and Chase suggested that the variation observed in the data may be a result of random variation and not a result of the treatment.

Dr. Sobol explained that the proposed study will provide definitive information about antitumor activity because of the number of patients who will be accrued. Data derived from this larger study will yield statistically significant results. The proposed study will provide useful information about autologous versus allogenic immunization. Allogeneic stimulation would provide a less costly approach to this treatment.

In response to Dr. Parkman's question about the allogeneic cell line, Dr. Sobol explained that this tumor cell line was established from the patient treated on the previous trial. This allogeneic cell line will be transduced with a different retrovirus vector than was used for the previous trial. The new vector will produce higher levels of IL-2.

With regard to the *in vitro* data, Dr. Straus asked if the variation in CTL activity and the clinical responses observed could be a result of the other concurrent therapies (e.g., steroids) the patient was receiving. Dr. Sobol answered that although the patient received prolonged Decadron treatment, the antitumor activity and clinical changes could be a result of high IL-2 expression by the transduction of cells. Dr. Straus disagreed with the investigator's interpretation of these results. Dr. Miller stated that these preliminary data are insufficient to support the proposal because the results are not interpretable.

Dr. Carmen stated that the NIH Director should never have permitted this single patient protocol to be performed. No scientifically valid data can be derived from this single patient protocol. The investigators failed to provide evidence of preclinical efficacy in an appropriate animal model.

Committee Motion

A motion was made by Dr. Carmen and seconded by Dr. Brinckerhoff to disapprove the protocol. Dr. Post remarked that a relevant animal model is not available for human brain cancer. Dr. Sobol said some *in vivo* experiments had been performed in which rats were immunized with glial tumor cells modified to produce IL-2; however, no antitumor effects were observed. Dr. Straus noted that the investigators have made a great effort to propose an experiment designed to treat a very desperate disease; however, the protocol lacks sufficient preclinical data to provide a scientific basis for the study. For these desperate patients, many therapies are given to patients that complicate the study. If these alternative treatments are withheld from patients because of some false promise of gene therapy, the investigators could harm the patients. Lacking other supportive studies, Dr. Straus recommended disapproval of this study.

Dr. Parkman explained that he would not vote for disapproving this protocol. The RAC has approved other protocols with similar preclinical data. Since the investigators propose to use lethally irradiated cells, there is minimal risk associated with the experiment. The potential risk to patients is that other forms of therapy may be withheld from these patients.

Dr. Haselkorn stated that there are other forms of harm that could occur as a result of a patient's participation in this study such as psychological harm to the patient's family based on false expectations and discrediting the reputation of the medical research community because of misrepresentation of data (i.e., the Reuters new report). Dr. Sobol responded that he does not have control over the press. Dr. Leventhal quoted a statement from a scientific abstract that was submitted by the investigators to the International Conference on Brain Tumor Research and Therapy 1993: "these encouraging results suggest that evaluation of this form of IL-2 gene therapy in additional patients with glioblastoma is warranted." Dr. Smith stated that immunological data obtained from the single patient trial are uninterpretable; however, the animal data provided in support of the investigators' colon cancer protocol should be considered acceptable preclinical data for the glioblastoma study. Immunological experiments could be redesigned to assess the baseline that would render the present data interpretable. Additional immunophenotype data are necessary.

Dr. DeLeon stated that the RAC should be objective and consistent in its review and approval of protocols. Since the investigators have provided *in vitro* data as well as *in vivo* data to support the colon carcinoma protocol, she recommends approval of this protocol. Dr. Krogstad said that this protocol could be strengthened to obtain interpretable data. Dr. Krogstad said that the RAC should consider approval of this study on the basis of the material submitted, and should not consider the confrontational circumstances in which the single patient protocol was approved.

The motion to disapprove the protocol passed by a vote of 10 in favor, 5 opposed and 1 abstention. The majority of the RAC members concluded that the preclinical data derived from a single patient protocol was inadequate to justify the proposal.

XI. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *RETROVIRUS-MEDIATED TRANSFER OF THE cDNA FOR HUMAN GLUCOCEREBROSIDASE INTO PERIPHERAL BLOOD REPOPULATING CELLS OF PATIENTS WITH GAUCHER'S DISEASE*/DR. SCHUENING

Review--Dr. Haselkorn

Dr. Walters called on Dr. Haselkorn to present his primary review of the protocol submitted by Dr. Friedrich Schuening of the Fred Hutchinson Cancer Research Center, Seattle, Washington. Dr. Haselkorn noted that the RAC has previously reviewed and approved other gene transfer protocols for the treatment of Gaucher's disease. Gaucher's disease is caused by a genetic defect in which the lack of glucocerebrosidase

(GC) production results in the accumulation of glucocerebrosides. Current therapy consists of periodic injections of the purified human enzyme. This enzyme is found principally in small amounts in the placenta. The cost of treatment for a typical affected adult approaches $400,000 per year. Since enzyme replacement therapy has been demonstrated to alleviate symptoms in Gaucher patients, gene therapy is a logical next approach. The investigators propose to collect autologous peripheral blood cells, isolate CD34(+) stem cells (which are capable of repopulating bone marrow), and transduce these cells with a retroviral vector containing the human GC gene. These transduced cells will then be reinfused into the patient. Dr. Haselkorn asked the investigators to provide additional information about the level of GC expression by transduced CD34(+) cells. How does the level of GC expression observed by the investigators compare to the levels of expression observed by Drs. Barranger (University of Pittsburgh) and Karlsson (NIH)? Have experiments been performed comparing the proposed vector with the other two RAC-approved GC vectors? Commercial considerations should be ignored so that the optimum vector is proposed for all trials. Dr. Haselkorn expressed concern about the RAC's recommendation for approval of 3 simultaneous trials for the same disease. Preferably, the RAC should wait to obtain results from the ongoing studies before approving additional trials. Dr. Haselkorn noted that Dr. Schuening has not accrued any patients onto either of his previously approved trials for breast cancer and lymphoid malignancies.

Review--Dr. Brinckerhoff

Dr. Brinckerhoff stated that the current forms of therapy for Gaucher's disease, enzyme replacement and bone marrow transplantation, have shortcomings. Therefore, there is significant rationale for proposing gene therapy as a treatment for this disease. Although the investigators have conducted several preliminary *in vivo* and *in vitro* experiments, the data are diffuse and inconclusive. Dr. Brinckerhoff asked about the level of gene expression necessary to demonstrate efficacy in the humans. Although the investigators have demonstrated GC gene expression in fibroblasts, it is unclear how this data extrapolates to humans. Preliminary data does not demonstrate the duration and level of GC expression. Although long-term expression of the neomycin resistance (neo^R) gene was demonstrated using the proposed vector, the investigators were unable to co-transfect the ADA gene. Data has not been provided demonstrating long-term GC expression. Although the murine data were encouraging, the canine experiments were inadequate. Quality assurance data on the modified retrovirus vector, LgGC, and the packaging cells are inadequate. Is a 15 to 20% correction of the GC enzyme activity in these patients sufficient to alleviate the clinical symptoms? Will the investigators be able to achieve the proposed level of gene expression? Dr. Brinckerhoff recommended deferral of this protocol until the RAC has had the opportunity to review additional expression data.

Review--Dr. Carmen

Dr. Carmen stated that the only novel feature about this study over the previously approved Gaucher's disease protocols is that a new retrovirus vector, LgGC, is being

proposed. LgGC has been safety modified to reduce the risk of RCR. The investigators propose to use a new packaging cell line, PG13, to enhance gene expression. Preclinical safety testing data has not been submitted for this LgGC/PG13 system; however, the investigators note that similar data was not required for approval of Dr. Barranger's protocol of Gaucher's disease. Dr. Carmen recommended that the RAC maintain consistency in its review and approval of similar protocols. If accelerated review is adopted by the RAC, this protocol is an example of experiments that may qualify. In this particular situation, the onus would be on the investigator to provide satisfactory preclinical safety testing data for the new vector. He submitted several recommendations to the Informed Consent document to make the document more understandable to laypersons.

Other Comments

Dr. Parkman noted his intention to abstain from voting on this protocol due to conflict of interest (participated in a similar protocol at the University of Southern California). He commented that there may be a benefit to having 3 simultaneous trials for the same disease but utilizing 3 slightly different vector constructs. Biologically relevant information would be obtained in a shorter time frame. He expressed concern about whether data is conclusive about GC expression in hematopoietic stem cells, despite demonstration of such expression in other cell types such as fibroblasts. Dr. Haselkorn expressed concern about Dr. Schuening's inability to obtain adequate levels of GC expression using one of the other vectors previously approved by the RAC. Dr. Haselkorn noted that it was important for the PIs of these similar trials to maintain communication with other investigators about scientifically relevant data. Ms. Grossman stated that perhaps the investigators' failure to reproduce previous experiments is due to lack of experience with particular techniques. Dr. Parkman explained that expertise is a key element that must be considered especially when the RAC begins to consider multi-center trials. Investigators must demonstrate successful transduction even when the same vector and target cells are proposed.

Dr. Post asked the investigators to provide additional information about RCR testing of the LgGC/PG13 system. A new *env* gene has been introduced into this system.

Investigator Responses--Dr. Schuening

In response to the RAC's concerns about the choice of vector, Dr. Schuening said that he has collaborated extensively with Dr. Barranger on these preclinical studies. In regard to the issue of the optimal vector for stem cell transduction, the human clinical trials will most adequately address this question. Both the MFG-GC vector (used by Dr. Barranger) and the LgGC vector yield similar results with regard to *in vitro* transduction of human progenitor cells. Dr. Schuening clarified an earlier statement about the inability to reproduce similar levels of GC expression with Dr. Karlsson's vector. He was referring to the transduction experiment in which stromal monolayers were employed to maintain long-term repopulating cells. No patients have been accrued onto Dr. Karlsson's previously approved protocol because he is awaiting FDA approval. Dr.

Leventhal remarked that Dr. Schuening's semi-annual data report form included a statement that the reason for closure of the protocol on Hodgkin's disease was due to reported toxicities associated with IL-3. Dr. Schuening responded that a subsequent minor modification has been submitted in which permission is requested to replace IL-3 with the less toxic fusion protein, GM-CSF/IL-3, for mobilization of CD34(+) cells in the peripheral blood from bone marrow. Review of this minor modification is in progress.

In response to Dr. Brinckerhoff's question about the preclinical studies, Dr. Schuening explained that he has performed numerous experiments (*in vitro* and *in vivo*) using the proposed vector. The experiments have included small animals, large animals, established cell lines, and normal human hematopoietic progenitor cells. Data demonstrates that the GC enzyme levels in normal cells are increased above endogenous levels. These levels of increase are even higher in fibroblasts obtained from Gaucher patients, e.g., 12- to 18-fold over nontransduced controls. With regard to long-term animal data, canine experiments have demonstrated expression of neo^R out to 4 years following transplantation of transduced marrow cells. The inability to detect the co-transfected ADA gene in these animals was due to the high level of endogenous ADA activity.

Responding to Dr. Carmen's question about preclinical canine safety data, Dr. Schuening said that two animals have received LgGC transduced marrow cells and have been observed for approximately 6 months. No evidence of toxicity has been observed.

Dr. Miller stated that, as a collaborator on this protocol, he would respond to the RAC's questions about the retrovirus construct and packaging cell line. It is difficult to conduct comparison studies between investigators using different vector suppliers because of commercial considerations. Development and validation of vectors is extremely costly for the companies involved. The 3 GC vectors reviewed by the RAC are more similar than different. All of these vectors are all based on the Moloney murine leukemia virus (MoMuLV). The LgGC vector has a modified t-RNA binding site that eliminates the problem associated with expression by protein binding. Preliminary data indicate that this modified binding site allows the vector to express the gene insert better in fibroblasts than other vectors; however, there is no definitive data about this benefit in bone marrow expression to date. However, *in vitro* transduction of human bone marrow cells with the LgGC vector demonstrates increased GC activity, which is an indicator of gene expression.

Dr. Tom Reynolds from Targeted Genetics Corporation, Seattle, Washington, explained the RCR testing procedures. The most likely mechanism for generating RCR is homologous recombination between the vector and the retroviral genes inserted in the packaging cells. The LgGC vector lacks the retroviral *env* gene, and the PG13 packaging cells were made by independent introduction of the *gag-pol* genes of MoMuLV and the *env* gene of gibbon ape leukemia virus (GALV) into the NIH3T3 TK^- cells. These modifications were introduced to reduce the probability of generating RCR by recombination between the *env* genes of the vector and the packaging cells. Targeted

Genetics Corporation is developing improved assays to detect potential GALV recombinants. The human HeLa cell line, which is susceptible to GALV infection, will be used as a rescue cell line. Upon infection of the HeLa cells with RCR, the rescued vector is detectable by its hygromycin selectable marker. The sensitivity of this rescue assay is currently being determined. Dr. Miller explained that S^+L^- assays have been performed, and there is no evidence of RCR using the PG13 cell line. Dr. Post suggested that approval of this protocol might be contingent on the submission of RCR sensitivity assays.

Ms. Meyers asked whether recommendations for long-term follow-up and contraception for men/women were included in the Informed Consent document. Dr. Schuening replied that these issues have been addressed in the document. Dr. Parkman inquired about the length of time necessary for contraception to be practiced. If the experiment is successful, the inserted gene will persist during the life of these patients. Dr. Zallen said that it is reasonable to require contraception during the active phase of the gene transfer protocol.

Committee Motion

A motion was made by Dr. Haselkorn and seconded by Dr. Carmen to approve the protocol contingent on the submission of data demonstrating the level of sensitivity of assays for RCR and review and approval of this data by the primary reviewers. The motion to approve the protocol passed by a vote of 13 in favor, 0 opposed, and 3 abstentions.

XII. DISCUSSION ON INFORMED CONSENT ISSUES/DR. ELLIS

Dr. Zallen, Chair of the RAC Working Group on Informed Consent, presented an overview of the written questions that were forwarded to Dr. Gary Ellis, Director of the Office for Protection from Research Risks (OPRR) of NIH, prior to this meeting. She thanked Dr. Ellis for his willingness to present an oral response to the working group's questions.

Dr. Zallen explained that the working group focused its concerns in three areas: (1) the role of OPRR and its mechanism for oversight of local IRBs, (2) the relationship between IRBs and PIs (e.g., quality control for Informed Consent documents), and (3) the independent responsibilities and cooperative efforts of the RAC, OPRR, and IRBs, particularly in relation to human gene transfer. The RAC frequently encounters resistance from local IRBs concerning recommended changes to Informed Consent documents. Dr. Zallen inquired whether the RAC, as part of its advisory role, can condition its approval of a given protocol contingent on Informed Consent document changes. Dr. Zallen asked Dr. Ellis for recommendations on how to resolve issues in which there is disagreement between the RAC and the local IRB.

Dr. Ellis explained that OPRR is an office within the Department of Health and Human Services, but housed at NIH. OPRR oversees implementation of the federal regulations

for the protection of human subjects and oversees approximately 1,000 IRBs in the United States. OPRR discharges its responsibility through a process of assurance negotiation. For large institutions, multiple project assurances are franchised by OPRR. Each institution is responsible for the review of all Informed Consent documents and oversight of the consent process. For smaller institutions, the local IRBs work in tandem with the OPRR through single project assurances. The only Informed Consent documents routinely reviewed by OPRR are those from small institutions.

Dr. Ellis said that a very important component of the oversight system is vesting the IRB with the authority for oversight of the Informed Consent process at the local level since the IRB is in the best position to reflect the local and state laws, institutional policies and responsibilities, and diversities in patient population. OPRR provides informal guidance through periodic contact with the institution, informational conferences, and through the newsletter, *OPRR Reports*.

OPRR assures compliance through a formal mechanism. The Federal regulations include eight elements that must be considered when drafting an Informed Consent document. The RAC can forward recommendations (e.g., specific to gene transfer) to OPRR for inclusion in Informed Consent documents. If endorsed by OPRR, the recommendation would be transmitted to the local IRBs through *OPRR Reports*. Dr. Ellis suggested the RAC could amend the *Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects (Points to Consider)* to include pertinent issues that need to be addressed in the Informed Consent document and would work in tandem with the IRBs to educate investigators with regard to the preparation of Informed Consent documents for human gene transfer protocols.

Ms. Meyers asked who has the final authority over Informed Consent documents, the RAC or the IRB. Dr. Ellis explained that while the IRB has purview over these documents, the RAC is in a position to recommend necessary changes. Dr. Zallen asked if the NIH Director has the authority to overrule the local IRB. Dr. Ellis said that the Director has ultimate authority over NIH research grant awards. Since the NIH Director can withdraw grant monies, IRB's tend to comply with NIH regulations and recommendations so their funds will not be jeopardized.

Dr. Parkman said that Dr. Ellis has suggested a very clear two-prong approach with regard to Informed Consent documents; therefore, the working group should now be charged with developing *suggested* language for recommendation to OPRR and the *Points to Consider* document.

A lengthy discussion ensued about compensation for costs associated with research-related injuries, an issue that frequently remains unresolved between the RAC and local IRBs. Dr. Ellis agreed that there are serious ethical considerations if patients were required to pay for such costs; however, these issues are beyond the scope of OPRR. The issue of compensation for research-related injury has previously been addressed by the RAC and has been brought to the attention of the Health Care Reform Task Force

of the Clinton Administration.

In conclusion, most RAC members agreed with Dr. Ellis' suggestion that the RAC should draft a letter outlining the specific recommendations to the OPRR for consideration and distribution to local IRBs as well as proposed amendments to the *Points to Consider.* The working group should develop language that addresses the following: (1) recommendations for contraception by males/females, (2) responsibility for financial costs of the experiment, (3) the necessity for long-term follow-up, (4) request for autopsy, and (5) protection of patient confidentiality when information is released to the media.

XIII. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *AN OPEN LABEL, PHASE I/II CLINICAL TRIAL TO EVALUATE THE SAFETY AND BIOLOGICAL ACTIVITY OF HIV-IT(V) (HIV-1 IIBenv/RETROVIRAL VECTOR) IN HIV-1 INFECTED SUBJECTS*/DR. HAUBRICH

Review--Dr. Straus

Dr. Walters called on Dr. Straus to present his primary review of the protocol submitted by Dr. Richard Haubrich of the University of California at San Diego Treatment Center, San Diego, California. The primary objectives of this protocol are to evaluate safety and to ascertain the immunological effects of the human immunodeficiency virus (HIV)-IT(V) vector in HIV-1(+) asymptomatic individuals. The treatment is designed to stimulate a CTL response against the HIV-1 *env/rev* proteins. This response could lead to a reduction in the number or elimination of HIV-infected cells. Enhanced viral clearance could reverse immunosuppression or inhibit its progression. This proposal represents an extension of Dr. Galpin's previously approved protocol in which a murine retroviral vector containing HIV-1 *rev* and *env* genes was injected intramuscularly into HIV(+) subjects. This current proposal incorporates the following modifications as compared to Dr. Galpin's protocol: (1) patient eligibility will be expanded to include patients with CD4 counts between 200 and 499, (2) the highest virus inoculum proposed for this study is 10^7 cfu, (3) doses will be administered to multiple muscle sites, (4) doses will be administered at 3 monthly intervals rather than biweekly intervals, and (5) antiviral therapy will be permitted but withheld for 3 days prior to and 3 days following vector injection. The vector, study rationale, and the preclinical data are well presented. The Informed Consent document is acceptable with the inclusion of modifications relating to request for autopsy and long-term follow-up. There are still several outstanding issues that should be addressed by the investigators. The current proposal is an extension of a previous study from which data is not yet available. The investigators should verify that the previous doses, including the 10^7 cfu proposed for this study, have been well tolerated. The investigators should explain the rationale for proposing multiple injection sites, i.e., was a single injection site inadequate to confer a maximal response? Even though no interpretable data has been reported for the Phase I study, the investigators are requesting expansion to a Phase II trial. Although the vector may be safe, the RAC should carefully consider accelerated transition through these phases.

The Informed Consent document contains an awkward and misleading statement about injuries arising in the course of the study will be covered except when they are "a consequence of research procedures designed directly to benefit..." the subject. Since this study may offer the potential of benefit, this statement could be interpreted that no coverage would be forthcoming. The investigator has provided a thorough response and a summary of the clinical experience with the 12 patients treated. No serious adverse effects were observed at any dose. In his written response, Dr. Haubrich states that this Phase I/II trial will not be initiated until the 12 subjects enrolled in the existing Phase I study have completed a full course of HIV-IT(V) or placebo injections. Preliminary data indicate that this treatment is safe; therefore, the protocol should be approved.

Review--Dr. Dronamraju (presented by Dr. Walters)

Dr. Walters summarized Dr. Dronamraju's written review. Although the animal data justifies this protocol, data has not been provided from the ongoing human study. The total number of patients accrued onto each stratum of the study should be clarified. The Informed Consent document acronyms should be revised so that terms such as CTL and HIV-IT(V) are clearly defined and are comprehensible to laypersons. The Informed Consent document should include a request for autopsy. He inquired whether the "Experimental Subject's Bill of Rights" is a standard component of all protocols at the institution.

Review--Dr. Secundy (presented by Dr. Walters)

Dr. Walters summarized Dr. Secundy's written comments. The data from the previous human study is insufficient with regard to CTL activity, antibody response, viral burden, and possible adverse effects. How long were the murine and baboon experiments carried out? What mechanisms are in place to ensure access to this study by women and minorities?

Other Comments

Dr. Carmen said that the Informed Consent document is unacceptable in its present form and submitted specific changes in writing that would make the document more comprehensible to laypersons. He inquired whether the patient information brochure prepared for Dr. Galpin's study would be given to patients considering participation in this study. Dr. Parkman inquired about the length of time the vector sequences will persist at the injection sites.

Ms. Meyers stated that the Informed Consent document is very poorly written. Approval of this protocol should be contingent on review and approval of a revised Informed Consent document. Ms. Meyers agreed with Dr. Straus' interpretation of the compensation for research-related injury clause. This statement should be deleted from the Informed Consent document.

Discussion ensued about how the RAC could relay its concerns to Dr. Haubrich's IRB

about the Informed Consent statements concerning compensation for research-related injury. Dr. Krogstad suggested that the protocol should be deferred with the stipulation that university representatives and/or the IRB Chair be present at the RAC meeting when the protocol is resubmitted for review. Dr. Parkman remarked that the present proposal is a Phase I/II trial that by definition is not for the benefit of the patient because there is no demonstration of efficacy. The sentence regarding benefits in the Informed Consent is not operative in this trial. Dr. Leventhal added that even Phase III studies are considered experimental and may not necessarily benefit the patient. Dr. Leventhal suggested that the RAC send a letter to Dr. Haubrich's IRB requesting that the statement about research-related injury be deleted from the Informed Consent document.

Mr. G'dali Braverman of ACT-UP applauded the investigators for modifying the inclusion criterion concerning lower CD4 counts thereby allowing more patients to be eligible for this study than the previously approved protocol. He commented on several other inclusion/exclusion criteria from a patient's point of view, such as cross participation in other studies, lowering the eligibility age from 18 to 13, accrual of women and minorities, use of antivirals, mentioning of other experimental protocols to patients and other minor points. Mr. Braverman recommended approval of the protocol if the RAC members' comments and questions are adequately addressed.

Investigators' Responses--Drs. Merchant and Haubrich

Dr. Bruce Merchant, Director of Clinical and Regulatory Affairs, Viagene, Inc., San Diego, California, responded to the RAC's questions regarding the retrovirus vector. Murine data indicates that the vector sequences are detectable out to 56 days at the site of the injection. No sequences were detectable in the ovaries, lungs, lymph nodes, spleen, or liver of either primates or mice during this period. No vector sequences were present in primate sperm; however, data is not yet available about viral sequences at the site of injection in this group of animals. Any patients considering participation in the proposed study will receive the patient information brochure (same as for Dr. Galpin's study) several days in advance of the study coordinator interview. The Informed Consent document will be signed by the patient only after the brochure has been read and discussed with the coordinator. In regard to compensation for research-related injury, Viagene would be responsible for any expenses involved in the event of research-related injury. This policy is clearly stated in the patient information brochure; however, the IRB requested that this statement be removed from the Informed Consent document. Dr. Merchant encouraged the RAC to send a recommendation about this issue to the IRB. He agreed to include the Informed Consent document changes suggested by the RAC and expressed his appreciation for Mr. Braverman's comments and suggestions.

Discussion

There was a brief discussion regarding whether the letter to the IRB should be a contingency for approval of the protocol. Dr. Straus said that a stronger message would be sent to the IRB if this letter was a contingency. He said that the letter can be sent

out from ORDA. Dr. Chase recommended that the RAC take stronger action concerning the issue of research injury compensation. Dr. Zallen remarked that the Informed Consent review should be part of the protocol approval process and agreed that a letter should be sent to the IRB as a contingency for approval.

Dr. Parkman remarked that if the present treatment has no benefit to the patients, the statement on compensation for treatment intended to benefit is not operative. Dr. Merchant agreed to include a statement in the Informed Consent to indicate that the present treatment has no known or expected benefit to patients.

Committee Motion

A motion was made by Dr. Straus and seconded by Ms. Meyers to approve the protocol with the contingency that the primary reviewers review and approve the following: (1) a revised Informed Consent document which includes changes suggested by the RAC and includes the *Participant Information* brochure as an Appendix; (2) the Informed Consent document should be revised to include the statement, "This treatment has no known benefit, and there is no expectation that there will be any benefit to you," and (3) a letter should be sent to the IRB of the University of California at San Diego by ORDA requesting the following paragraph be deleted from the Informed Consent document:

> "If I am injured as a result of participation in this research, the University of California will provide any medical care I need to treat those injuries - except when they are a consequence of research procedures designed to benefit me directly. The University will not provide any other form of compensation to me if I am injured."

The motion to approve the protocol passed by a vote of 15 in favor, 1 opposed, and 2 abstentions.

XIV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I TRIAL OF B7-TRANSFECTED LETHALLY IRRADIATED ALLOGENEIC MELANOMA CELL LINES TO INDUCE CELL-MEDIATED IMMUNITY AGAINST TUMOR-ASSOCIATED ANTIGENS PRESENTED BY HLA-A2 OR HLA-A1 IN PATIENTS WITH STAGE IV MELANOMA*/DR. SZNOL

Review--Dr. Parkman

Dr. Walters called on Dr. Parkman to present his primary review of the protocol submitted by Dr. Mario Sznol of the NIH, Frederick, Maryland. Dr. Parkman explained that the investigators propose to enhance the immunogenicity of tumor cells by introducing the B7 gene into tumor cells lacking this antigen. Two signals are required to induce CTL activity. One signal is initiated when the melanoma antigen binds to the T cell receptor. The second signal originates from the binding of the B7 antigen to the CD28 receptor of T cells. Melanoma cells are resistant to CTL killing because these

cells do not express this secondary B7 signal. The investigators propose to introduce the B7 gene into melanoma cell lines to enhance immunogenicity. Preclinical *in vivo* data indicates introduction of the B7 gene into non-immunogenic tumor cells enhances tumor cell immunogenicity and induces CTL activity, which results in rejection of both B7-modified and non-modified tumor cells. As compared to cytokine gene transfer protocols, this protocol has no possibility of adverse effects associated with systemic cytokine toxicity. As a point of clarification, Dr. Parkman explained that the B7 antigen proposed for this study is different from the HLA-B7 antigen previously approved by the RAC for Dr. Gary Nabel's protocol (University of Michigan #9306-045). B7 is not an HLA antigen, but belongs to a group of accessory molecules that bind to the CD28 receptor.

Dr. Parkman explained that the patient population will be limited to either HLA-A1 or HLA-A2 individuals to ensure the appropriate HLA match with the corresponding melanoma cell lines. Approximately 50 patients will be divided into 3 dose escalation groups to assess toxicity. Each group will receive 6 injections of 10^7, 10^8, or 10^9 cells that have been lethally irradiated at 20,000 rads. The cDNA of the B7 gene will be expressed in the bovine papilloma virus (BPV) vector, BCMGNeo-B7. The B7 gene is regulated by a CMV promoter and the neo^R gene by the SV40 promoter. No other gene products will be expressed from this construct. This vector will be transfected into 3 melanoma cell lines using DNA-liposomes (lipofection). Only plasmid DNA will be used for the lipofection procedure; therefore, there is little risk of transmission of the transduced gene to host cells. High dose irradiation will render the tumor cells nonviable. Patients will be evaluated for immunological responses and evaluated for any toxicity associated with the transduced tumor cells.

Dr. Parkman inquired whether the HLA-A2 patients will receive injections of all 3 transduced melanoma cell lines on a rotating basis, and the HLA-A1 patients will receive only the HLA-A1/HLA-A2 cell line. Since 40% of Caucasians are HLA-A2, perhaps the protocol should be limited to this population in order to simplify interpretation of the data. He inquired about the necessity to accrue 50 patients on the proposed study. If a total of 6 patients are proposed for each dose group, 18 patients should be accrued. What is the rationale for injection of untransduced cells? Is there additional data demonstrating *in vitro* CTL responses? Since the scientific end point of this study is production of a CTL response, *in vitro* preclinical CTL data must be provided. The PI has submitted additional data in response to the written primary review; however, there are several remaining questions regarding specificity of cell killing and the HLA type or subtype of the normal donor cells.

Review--Ms. Grossman

Ms. Grossman noted that many of her concerns were addressed by Dr. Parkman. The preclinical data demonstrating *in vitro* CTL activity are inadequate, the vector sequence is inadequate, and there are no preclinical animal studies. She expressed concern that ectopic B7 expression might lead to the development of autoimmune disease due to immunogenicity to nontumor antigens. Based on the lack of scientific and immunologic

data, this protocol is thought to be premature and not be recommended for approval.

Dr. Parkman stated that scientific rationale for injection of untransduced cells was not well presented. This part of the protocol should be separated from the gene transfer protocol. Since the specificity of the CTL response has not been demonstrated, approval of the protocol is not justified.

Review--Dr. Zallen

Dr. Zallen questioned the scientific basis for treating HLA-A2 patients with a rotation of 3 different cell lines. She expressed concern about the Informed Consent process. Approximately 38% of the subjects recruited onto this study will later be informed that they are ineligible to participate based on the results of the HLA typing. How will the investigators deal with the emotional disappointment expected in these individuals? The protocol includes the statement "the financial costs of treatment are borne by the Biological Response Modifiers Program (BRMP) of the National Cancer Institute". However, this statement is not included in the Informed Consent document. The Informed Consent document should be revised to include requests for autopsy and long-term follow-up.

Other Comments

Dr. Geiduschek asked about the dilution of vector sequences following cell expansion. Since the BPV vector is non-replicating, will the vector be present after expansion of the transfected cells *in vitro*? Is the level of B7 expression stable in the clonal cell lines?

Dr. Leventhal agreed with Dr. Zallen's concern that the Informed Consent document does not properly inform subjects that they may be ineligible to participate in the study if determined not to be HLA-A2 or HLA-A1. Dr. Leventhal said that the Informed Consent document is too assertive for a Phase I experimental study and is unclear about how patients will be assigned into the control and experimental groups.

Dr. Carmen asked about Ms. Grossman's question about the incomplete vector sequence. A discussion ensued about the necessity to submit a complete sequence of every vector submitted for RAC review. Dr. Miller said that an assembled sequence is adequate for the current proposal. The investigators have demonstrated the functionality of the gene insert, the most important test for this construct. Since this gene construct is not proposed for a therapeutic purpose of correcting a gene deficiency, determination of the entire sequence of the gene insert is not essential. Insisting that the entire construct be sequenced would incur additional costs that are unnecessary. Dr. French Anderson of the University of Southern California noted that the *Points to Consider* require investigators to provide either a complete nucleotide sequence analysis or a detailed restriction enzyme map of the construct. There is no absolute requirement to totally sequence every gene construct.

Investigator's Responses--Drs. Fenton and Sznol

Dr. Robert Fenton of the National Cancer Institute, NIH, Bethesda, Maryland, explained that a substantial amount of information has been published in recent years regarding stimulation of immune responses by the B7 antigen. Dr. Fenton said that these published murine studies are the preclinical basis for the present human proposal. Although *in vitro* data demonstrating CTL activity was not submitted to the RAC, it was noted that these data are probably not critical for RAC review. With regard to B7 expression in transfected cell lines, only the highest B7-expressing clones were chosen. The vector sequences of these cloned cells were integrated into chromosomes at a rate of approximately one to two copies per cell. B7 expression is stable in these cell lines out to 14 days following lethal irradiation. Regarding the *in vitro* CTL activity, preliminary data indicates melanoma-specific CTL activity. Peripheral blood cells obtained from HLA-A2 melanoma patients were mixed with a rotating panel of 3 transfected cell lines for 6 weeks. Specific CTL activity was demonstrated toward melanoma cells. All donor cells have been typed for HLA.

Dr. Fenton explained that the 3 transfected melanoma cell lines will be rotated to minimize the risk that patients will not be exposed to an important antigen and to specifically boost the A2 response. HLA-A1 patients will be included in this study to broaden patient eligibility. Patients will receive untransduced cells in order to serve as a control group for assessing the differences in B7 expression. The BPV vector proposed for this protocol is similar to the vector previously approved by the RAC for Dr. Podack's human gene transfer protocol, except that the early region of BPV has been deleted, which contains the transforming genes of this virus. As a consequence, the present vector should be safer than the previously approved BPV vector. This deletion renders the vector incompetent for episomal replication. The gene is expressed only when the vector is integrated and into the target cell chromosome. Most of the vector sequences, with the exception of approximately 20 bases at the junction points, are included in the assembled sequence. The B7 gene is functional and the likelihood that the construct presents any significant risk to patients is small.

Dr. Sznol explained that all costs associated with patient participation will be covered by his institution and agreed to work with his IRB to alter the statement about costs in the Informed Consent document. A request for autopsy was not included in the Informed Consent document because most patients who are accrued on this protocol will not die while participating in the experiment. However, if such a statement is required by the RAC, he will include the revision in the Informed Consent document. Dr. Straus commented that although an autopsy is often difficult to obtain, an autopsy is preferred due to the special concerns that are unique to gene therapy. It is important to demonstrate that the gene sequences did not persist in any tissues. Ms. Meyers said that several standard items are missing from the Informed Consent document, e.g., request for autopsy, request for long-term follow-up, and recommendations for male/female contraception. Dr. Leventhal remarked that most patients will probably die at a distant location; therefore, autopsy requests should be relayed to local physicians. Dr. Sznol agreed to incorporate the RAC's suggested changes into a revised Informed Consent document.

Committee Motion #1

A motion was made by Ms. Grossman and seconded by Ms. Meyers to defer approval of the protocol on the basis of inadequate preclinical data.

Dr. Miller agreed with the investigators' assessment, that published studies adequately address the preclinical issues. Additional animal experiments will not add any new scientific information. Dr. Parkman said that his principal concern is that the end point of this study is to explicitly demonstrate melanoma-specific CTL responses; the data inadequately assesses the investigators' competence in performing these assays. Although the investigator's noted the existence of such data during their oral responses to the RAC, the data has not been submitted. Therefore, approval of the protocol should be deferred. Dr. Post said that published studies demonstrate the effect of B7 on tumor immunogenicity and provide strong justification for the present human study. Dr. Miller agreed with Dr. Post's assessment. Dr. Chase stated that he is inclined to defer this protocol. Dr. Geiduschek said that he is in favor of approving this protocol on the basis that the preclinical data in question is not critical to support this proposal. Drs. Haselkorn and Leventhal stated their concern that the RAC is employing a higher standard for this protocol than for previously approved studies; therefore, the protocol should be approved. Dr. Leventhal reminded the RAC that this protocol is a Phase I toxicity study; therefore, efficacy is not a primary objective. Dr. Straus said that the scientific background for this human trial is compelling and recommends approval.

The motion to defer the protocol did not pass by a vote of 5 in favor, 12 opposed, and 1 abstention.

Committee Motion #2

A motion was made by Dr. Post and seconded by Dr. Secundy to approve the protocol. Approval of the protocol is contingent on submission of the following: (1) data obtained from ongoing *in vitro* human melanoma experiments (to be reviewed by Dr. Parkman, but approval not required), and (2) inclusion in the Informed Consent document of a request for autopsy, a description of long-term follow-up, and a statement explaining that the study is non-beneficial (review and approval to be done by Drs. Leventhal and Zallen). A friendly amendment was made by Dr. Parkman and accepted by Drs. Post and Secundy to require that the Informed Consent document be separated into a document for subjects receiving transduced cells and another document for subjects receiving untransduced cells. The motion to approve the protocol passed by a vote of 15 in favor, 2 opposed, and 1 abstention.

XV. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *PHASE I STUDY OF IMMUNOTHERAPY OF ADVANCED COLORECTAL CARCINOMA BY DIRECT GENE TRANSFER INTO HEPATIC METASTASES*/DR. RUBIN

Review--Dr. Doi

Dr. Walters called on Dr. Doi to present his primary review of the protocol submitted by Dr. Joseph Rubin of the Mayo Clinic, Rochester, Minnesota. Dr. Doi stated that the primary objective of this study is to determine the safety and feasibility of the direct injection of DNA/lipid complexes into hepatic metastases of patients with advanced colorectal carcinoma. This protocol is similar to Dr. Nabel's protocol that was previously approved by the RAC at its June 1993 meeting. There is no increased risk to patients in the present protocol, and the present approach is even more conservative than that of Nabel's. The investigators will attempt to stimulate an *in vivo* immune response by direct intratumoral injection of lipid complexes containing genes encoding the HLA-B7 histocompatibility antigen and ß-2 microglobulin. *In vivo* data demonstrates the attenuation of tumor growth; and in some instances, complete tumor regression as a result of this treatment. The DNA of the plasmid vector, pHLA-B7/ß-2, will be mixed with the cationic lipid, DMRIE (1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl ammonium bromide), and the neutral lipid, DOPE (dioleoylphosphatidylethanolamine). These DNA/lipid complexes will be injected into the tumor by a thin needle guided by sonography. The procedure has an accuracy rate of 98%. The injection sites will be visualized and documented on videotape. Approximately 1% of cells within the tumor mass will be transfected. A total of 15 HLA-B7(-) colorectal patients with hepatic metastases will be treated in dose-escalation groups to determine toxicity. Patients will be divided into two treatment schedules. Toxicity symptoms such as fatigue, weight loss, nausea, vomiting, hemorrhage, infection, and liver chemistries will be evaluated during the test period and immunologic responses will be monitored. Most of the safety issues have been addressed. Although there is no control group in the present study and no clear description about the immune responses that will be monitored, Dr. Doi recommended approval of the protocol.

Review--Dr. DeLeon

Dr. DeLeon said that this protocol is a conservative revision of Dr. Nabel's previously approved protocol. She noted some inconsistency in the number of hepatic injections between the Informed Consent document and the protocol. However, the investigators have clarified this issue. A request for autopsy should be included in the Informed Consent document. Dr. DeLeon recommended approval of the protocol.

Review--Mr. Capron (presented by Dr. DeLeon)

Dr. DeLeon provided an overview of Mr. Capron's written review to the Informed Consent document. The term "therapy" should not be used because the investigational nature of the study is not adequately conveyed. Suggested language was submitted that would improve comprehension by laypersons.

Other Comments

Dr. Leventhal asked the investigators to compare the doses of DNA to those administered in Dr. Nabel's protocol. Ms. Meyers raised several concerns about the Informed Consent document, i.e., long-term follow-up, recommendations for

contraception, and request for autopsy. Ms. Meyers asked the investigators to clarify the patient's responsibility for any costs related to the treatment.

Investigator Response--Dr. Kovach

Dr. John Kovach of the Mayo Clinic, Rochester, Minnesota, said that the major end point of this study is any observed change in the titer of anti-B7 CTL activity in the peripheral blood. Although the investigators have to resolve certain technical difficulties involving assays of biopsy materials, specific cytotoxicity of the untransduced tumor cells will be determined. With regard to the Informed Consent document issues, all patients will have life long follow-up, and none of the costs of the research will be charged to patients. Changes will be made to the Informed Consent document to clarify these points. The current statement about research-related injury is derived from other protocols previously reviewed by the RAC. A request for autopsy will be included in a revised document.

Dr. Alan Schreiber of Vical, Inc., San Diego, California, clarified the discrepancy of the current DNA doses to that of the Nabel protocol. Dr. Schreiber noted Vical's intention to initiate this protocol at other sites and encouraged the RAC to adopt an accelerated review mechanism for similar trials. Dr. Parkman said that multi-institution studies can be encompassed by the current review mechanism if such information is available at the time of submission for RAC review.

Committee Motion

A motion was made by Dr. DeLeon and seconded by Dr. Doi to approve the protocol with the stipulation that a revised Informed Consent document, including the changes suggested by the RAC, be reviewed and approved by the primary reviewers. The motion to approve the protocol passed by a vote of 18 in favor, 0 opposed, and no abstentions.

XVI. UPDATE ON DR. ROTH'S PROTOCOL ENTITLED: *CLINICAL PROTOCOL FOR MODIFICATION OF ONCOGENE AND TUMOR SUPPRESSOR GENE EXPRESSION IN NON-SMALL CELL LUNG CANCER*, #9209-031/DR. ROTH

Dr. Walters called on Dr. Miller to present an update on the protocol submitted by Dr. Jack Roth of MD Anderson Cancer Center, Houston, Texas, that was approved with contingencies at the September 1992 RAC meeting. Dr. Miller summarized the chronology of events that have transpired since September 1992. RAC approval of Dr. Roth's protocol was contingent on: (1) submission of data demonstrating the transforming potential of 100 ml of retroviral supernatant analogous to the preparation that will be used for the clinical protocol, (2) submission of data obtained from *in vitro* mixing experiments, (3) submission of *in vitro* data demonstrating that the new vector preparations have activity, and (4) incorporation of minor changes in the Informed Consent document as noted by Drs. Carmen and Hirano.

On May 11, 1993, Dr. Roth submitted data in response to the stipulations and requested

a minor modification to change the site of production of the clinical grade retroviral preparations from Genetic Therapy, Inc., to Microbiological Associates, Inc. After reviewing the submitted data, all three primary reviewers, Drs. Miller, Hirano, and Geiduschek, recommended disapproval. On September 22, 1993, and October 7, 1993, Dr. Roth submitted additional data. The three primary RAC reviewers subsequently recommended disapproval of this additional data submitted in response to the stipulation requirements, although the minor modification was approved. On November 11, 1993, Dr. Roth submitted additional data and made a request for a compassionate plea exemption. The request for compassionate plea exemption was denied by ORDA on November 16, 1993, based on the "Procedures to be Followed for Expedited Review" (58 FR 2174). Drs. Miller, Geiduschek, and Hirano did not accept the additional data as fulfilling the stipulation requirement. Dr. Miller suggested that the full RAC should discuss whether Dr. Roth's data adequately meets the stipulation requirements for approval. Upon request by Dr. Walters (Chair), Dr. Roth provided a written statement on December 1, 1993, providing his rationale that the stipulation requirements were adequately addressed.

Dr. Miller explained that there are concerns regarding safety and efficacy issues since this gene transfer protocol introduces oncogenes (which promote cancer development) and tumor suppressor genes (which retard tumor growth). The first stipulation was to provide data demonstrating lack of generation of transforming virus in 100 ml of the clinical grade retrovirus supernatants. Subsequent discussion between the primary reviewers and the investigator resulted in a modification of this stipulation. The revised stipulation is: "assay a single patient dose for the presence of transforming virus, i.e., 10 ml of supernatant at a vector titer of 1 x 10^7 cfu/ml (total of 10^8 cfu)." The data provided by the investigator in response to this stipulation was found to be inadequate due to lack of proper control experiments and the low level of sensitivity of the assay. The second stipulation involves the provision of data documenting the "bystander effect" claimed by the investigators, i.e., the ability of gene-modified tumor cells to suppress the growth of unmodified tumor cells. This "bystander effect" is crucial for efficacy of the present approach to suppress tumor growth, since only a small faction of tumor cells will be transduced by the vectors. Although there was an initial misunderstanding by the investigators with regard to this stipulation, Dr. Miller stated that he held extensive telephone conversations with Dr. Roth, in which the stipulation was explained and the investigator stated that he understood the necessary requirements. Dr. Miller noted that his comments with regard to this stipulation were outlined extensively in his review of this protocol. Data has never been submitted in response to this second stipulation. The third stipulation involves demonstration that there are no rearrangements in the vector structure during vector production from the producer cells. Dr. Miller stated that the new vector LNp53B, employs a *bidirectional* SV40 polyadenylation site that promotes rearrangement and may result in vectors with unknown activity. Northern and Southern blot analyses of the vector structure, and transcription in producer cells would demonstrate that there is no such rearrangement. The investigators have not provided satisfactory data in response to this third stipulation. The fourth stipulation involves minor changes in the Informed Consent document. An amended document was submitted by the investigators and adequately meets this stipulation.

Dr. Secundy inquired about the length of time required to perform the necessary experiments. Dr. Miller explained that the requested assays are relatively simple, taking only a few hours to set up, with several weeks of observation before the results are obtained. Time and cost are several reasons cited by Dr. Roth for not performing these experiments. Dr. Miller said that the investigator has stated that the protocol is justified on the basis that the proposed patient population is terminally ill. Dr. Chase said that this latter rationale is invalid. Dr. Walters stated that the consensus of the committee should be obtained with regard to recommendations for this protocol.

Dr. Post suggested that because of the lengthy delays that have occurred, the protocol should be resubmitted for reconsideration by the full RAC. Drs. DeLeon, Krogstad, and Ms. Grossman supported this suggestion. Dr. Geiduschek stated that a protocol cannot be considered approved by the RAC unless all stipulation requirements have been met. Dr. Leventhal suggested that the protocol should be resubmitted, and that different primary reviewers should be assigned. Dr. Parkman said that approval of this protocol should be considered administratively inactivated due to failure to meet the stipulation requirements.

Committee Consensus

The consensus of the RAC was that Dr. Roth should resubmit a revised protocol, including all additional data, for review and approval by the full RAC based on the following: (1) failure of the primary reviewers to recommend approval of the protocol, (2) lengthy delays that have occurred, (3) there are several new members on the RAC who were not on the committee at the time the original protocol was reviewed, and (4) Dr. Roth has requested the use of a substitute vector. If Dr. Roth submits a revised protocol, new primary reviewers will be assigned. The consensus of the RAC is that the protocol is considered administratively inactivated; therefore, RAC approval of the protocol is withdrawn. The RAC recommended that ORDA forward a letter to Dr. Roth outlining the consensus of the RAC.

XVII. ADDITION OF APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *ADOPTIVE IMMUNOTHERAPY OF MELANOMA WITH ACTIVATED LYMPH NODE CELLS PRIMED IN VIVO WITH AUTOLOGOUS TUMOR CELLS TRANSDUCED WITH THE IL-4 GENE*/DR. CHANG

Review--Dr. Geiduschek

Dr. Walters called on Dr. Geiduschek to present his primary review of the protocol submitted by Dr. Alfred E. Chang of the University of Michigan, Ann Arbor, Michigan. Dr. Geiduschek explained that this protocol is an extension of an ongoing non-gene transfer adoptive immunotherapy protocol for renal cell carcinoma and melanoma in which patients are vaccinated with their own tumor cells that have been lethally irradiated, mixed with BCG (Bacille Calmette-Guerin) adjuvant, and injected in the vicinity of lymph nodes, which are subsequently removed. Lymphocytes from these excised lymph nodes are stimulated by exposure to a monoclonal anti-CD3 antibody and

expanded in the presence of IL-2 to yield a large population of antitumor effector T cells. The latter are reintroduced into patients with concurrent IL-2 administration. Dr. Chang reported a significant response to this procedure in renal cell carcinoma patients with a lesser response observed in melanoma patients. This gene transfer protocol is intended to address melanoma because of the decreased response to adoptive immunotherapy.

Dr. Geiduschek explained that this proposal attempts to up-regulate the immunogenicity of patients' tumor cells through enhanced IL-4 expression by transduction with the GBAH4-18 retroviral vector. Fifteen patients with advanced melanoma will be entered into this protocol. The objectives of this study are to: (1) assess the feasibility of transducing patients' tumor cells with the IL-4 gene and assess toxicity, (2) evaluate antitumor efficacy and *in vivo* immunological responses, and (3) evaluate the immunological reactivity of activated lymph node cells *in vitro*. There have been disappointing results obtained from the murine studies; no significant improvement was observed with IL-4 expressing cells. Dr. Geiduschek posed the following questions. What is the rationale for using the chicken actin promoter for expression of the IL-4 gene? Optimal and sustained IL-4 production has not been demonstrated using patient tumor cells. Is there *in vitro* data available that demonstrates the immunological reactivity of these transduced cells? Have any differences been observed between lymph node cells stimulated with untransduced tumor cells versus IL-4 transduced cells? Although the PI has responded to some of the concerns raised by the primary written review, the protocol is too premature to recommend approval. However, if the RAC does recommend approval of this protocol, there should be stipulations to address the technical shortcomings of the study.

Review--Dr. Motulsky (presented by Dr. Geiduschek)

Dr. Geiduschek summarized Dr. Motulsky's written comments. This approach has biologic plausibility and appears feasible. The treatment schema is complex and requires considerable manipulation of tumor cells, patient immunization, preparation of lymphocytes from lymph nodes, and IL-2 administration. Since the investigators have previously demonstrated success with an analogous non-gene therapy approach, this study is justified and could result in improved tumor therapy. The Informed Consent document is appropriate. Since there are no new risks associated with the gene manipulation aspects of this study, the protocol should be approved by the RAC.

Review--Ms. Meyers

Ms. Meyers' concerns focused primarily on the Informed Consent document. The investigators have adequately responded to initial concerns regarding the use of the term "tumor vaccine," recommendations for male/female contraception, and patients' responsibility for research-related costs; therefore, the revised Informed Consent document is acceptable.

Investigator's Responses--Drs. Chang and Kraus

Dr. Chang responded to the RAC's questions about preclinical data and stated that his co-investigator, Dr. Kraus, will address the RAC's questions about the proposed vector and transduction procedures.

Responding to the comments raised by Dr. Geiduschek, Dr. Chang said that in their own interpretation, the animal data demonstrates increased immunogenicity in response to IL-4 transduced lymphocytes compared to untransduced cells. Similar results have been obtained using another IL-4 expressing vector construct. Dr. Chang stated that Dr. Geiduschek's comparison between IL-4 and BCG is not pertinent for this human trial because a different and a more potent bacterial adjuvant, *C. parvum*, was used for the murine studies. Although the response with IL-4 transduced cells was similar to results obtained with *C. parvum*, IL-4 transduced cells were more effective than untransduced tumor cells alone. The animal data demonstrated an antitumor response for established tumors. For this reason, the animal studies provide sufficient justification for the proposed human clinical trials.

In response to Dr. Geiduschek's questions about immunological assays, Dr. Chang said that immune reactivity of draining lymph node cells will be determined by cytolytic activity, cell proliferation, and cytokine release. An autologous tumor cell delayed skin test will be used to determine the *in vivo* response.

Dr. Parkman inquired about the number of animals used for the animal studies. Dr. Chang said that an extensive murine experiment was conducted involving 70 mice (5 mice in each experimental group). This animal model is being used to define the most pertinent assays for the human study.

Dr. Geiduschek reiterated his reservations about the interpretation of the murine experiment. Since Dr. Chang has agreed that the experiment did not demonstrate an improved immune response using IL-4 transduced cells over the bacterial adjuvant, i.e., *C. parvum*, the animal data do not provide justification for the human study. Dr. Leventhal stated that the investigators should not be penalized for results that indicate that IL-4 therapy is equivalent to the best bacterial adjuvant therapy. Since IL-4 and BCG stimulate the immune system by different mechanisms, the combination of these two stimulants may yield a synergistic effect. Dr. Parkman cautioned that the animal data cannot be directly extrapolated to the human study. The relative potency of IL-4 and bacterial adjuvants may be different in humans; however, it is unnecessary to demonstrate that IL-4 is a more effective stimulant to justify the human study. Patients should have the option to choose the clinical protocol in which they desire to participate. Dr. Leventhal added that BCG and *C. parvum* are very complex bacterial adjuvants, and IL-4 is a less complex protein; therefore, scientific interpretation of the IL-4 data would be less complicated. Dr. Chang presented additional murine data to substantiate his assertion that IL-4 transduced cells elicit an enhanced immunologic response as compared to untransduced cells.

Dr. Kraus answered the RAC's questions about tumor cell transduction. Most of the experiments have been performed using late passage melanoma cells rather than early

passage cells, which are more relevant to the human study. Dr. Geiduschek suggested the RAC might condition its approval on optimization of the transduction efficiency and IL-4 production. Dr. Kraus agreed to accept a minimum production level of 50 picograms (pg) of IL-4/10^6 cells/ml/24 hours.

Dr. Leventhal suggested the inclusion of a stopping rule that if the investigators are unable to transduce cells at the minimum level of IL-4 production in 3 of the first 6 patients, the investigators are not permitted to treat any additional patients until they return to the full RAC for discussion of the data. Dr. Chang said that a range of IL-4 expression would allow for a dose-response assessment. Dr. Parkman responded that a 10-fold lower level of IL-4 expression resulted in antitumor responses in the animal model; therefore, the level of IL-4 secretion stipulated by Dr. Geiduschek is acceptable.

Committee Motion

A motion was made by Dr. Leventhal and seconded by Dr. Carmen to approve the protocol. Approval of the protocol is contingent on submission of the following: (1) data demonstrating optimization of cell transduction in early passage human melanoma cells and a minimum level of IL-4 secretion (≥ 50 pg of IL-4/10^6 cells/ml/24 hours), and (2) inclusion of a stop criterion that if the investigators are unable to transduce cells at the minimum level of IL-4 secretion in 3 of the first 6 patients enrolled in the study, the investigators will not be permitted to treat additional patients without returning to the full RAC for discussion of the data. The motion to approve the protocol passed by a vote of 12 in favor, 3 opposed, and 1 abstention.

XVIII. UPDATE ON THE HUMAN GENE TRANSFER PROTOCOL ENTITLED: *A PHASE I STUDY, IN CYSTIC FIBROSIS PATIENTS, OF THE SAFETY, TOXICITY, AND BIOLOGICAL EFFICACY OF A SINGLE ADMINISTRATION OF A REPLICATION-DEFICIENT RECOMBINANT ADENOVIRUS CARRYING THE cDNA OF THE NORMAL HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR GENE IN THE LUNG*/DR. CRYSTAL

Dr. Crystal presented a progress report on his ongoing CF protocol. He stated that the CFTR gene has been successfully delivered to the airway epithelium using an E1- and E3-deleted adenovirus vector. Both *in vitro* and *in vivo* expression of the CFTR gene has been demonstrated. Possible safety concerns are over-expression of the inserted gene, inflammation, immune reactions, generation of replication-competent virus, complementation, recombination, germ line transfer, and virus shedding. In animal studies involving rhesus monkeys, doses ranging between 10 and 100 times of those doses proposed for the human study demonstrated no acute or chronic clinical sequelae. Four patients have been treated on the human study to date. No adverse reactions were encountered with intranasal administration of the vector in any of these patients. These patients underwent delivery to the lower lobe of one side of the lung by a fiberoptic bronchoscope. One patient received a dose of 2 x 10^6 cfu, 2 patients received 2 x 10^7 cfu, and 1 patient received 2 x 10^9 cfu.

There was no evidence of shedding of replication-competent adenovirus from any of the treated patients. A dose-dependent induction of complement fixation antibodies was demonstrated; however, no induction of neutralizing antibodies was observed in treated individuals. Patient 2A, who received the highest concentration of vector, developed a mild reaction. This 24 year-old female received a dose of 2×10^7 cfu to the nasal epithelium, and 24 hours later she received 2×10^9 cfu to the right lower lobe bronchus. She exhibited symptoms of fatigue for 5 to 7 days, intermittent fever for 6 days, hypotension, hypoxemia, and lung infiltrate in the right lower and middle lobes. Although the vector was administered to the lower lobe, infiltrate in the right middle lobe was confirmed by a chest X-ray. All reactions were transient and disappeared after symptomatic treatment. The single adverse effect was probably due to vector-induced inflammation of the lung. Other possible causes have been eliminated, such as pathology of the disease itself, complication of bronchoscopy, contamination of the vector preparation, and complementation or recombination of vector with other adenovirus strains. No adverse reactions were observed in the preclinical animal studies.

The efficacy data are incomplete at this time. CFTR gene expression was demonstrated in the nasal epithelium; however, functional data demonstrating correction of the CFTR deficiency in the nasal epithelium are suggestive but less conclusive. The present data defines a range of toxicity that will allow for the design of future experiments. Lower starting doses will be initiated in order to explore a dose range that will prove to be efficacious.

Dr. Miller asked whether any adverse reactions were observed in the monkeys following repeat vector administration. Dr. Crystal responded that no responses were observed in monkeys. These reactions may be specific to CF patients whose lungs already have abnormalities. Dr. Crystal explained that the volume of the vector has been reduced from 20 ml to 5 ml for lung administration to avoid the possibility of alveolar inflammation. The adverse event was reported to the RAC, IRB, FDA, and other investigators conducting CF gene transfer trials. Similar dose adjustments have been made in other CF trials.

XIX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *GENE THERAPY FOR CYSTIC FIBROSIS USING CATIONIC LIPOSOME MEDIATED GENE TRANSFER: A PHASE I TRIAL OF SAFETY AND EFFICACY IN THE NASAL AIRWAY*/DRS. SORSCHER AND LOGAN

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review of the protocol submitted by Drs. Eric J. Sorscher and James L. Logan of the University of Alabama, Birmingham, Alabama. Dr. Post explained that the objective of this proposal is to evaluate cationic liposome-based delivery of the CFTR gene to nasal respiratory epithelia of CF patients. The nasal airway epithelium is an ideal model for gene transfer since this epithelium exhibits a CF bioelectric defect and is easily accessible for safety and efficacy studies.

The study will utilize 3 ascending dosages of CFTR cDNA. Each patient will receive CFTR/liposome administration to one nostril and a mock transfection (lipid with DNA lacking CFTR) of the contralateral nostril as a control. The proposed DNA delivery system differs from the other CF protocols previously reviewed by the RAC. As noted previously by Dr. Crystal, there may be limitations to using the adenovirus vector; therefore, it is reasonable to investigate more than one gene delivery approach. Another cationic liposome CFTR protocol has been already initiated in the United Kingdom. In his initial review, Dr. Post requested the investigators to provide safety or efficacy data using the proposed cationic liposome, DMRIE/DOPE. The majority of the preclinical data was obtained using another lipid, DOTMA/DOPE, N-[1-(2,3-dioleoyloxypropyl)]-3-trimethylammonium-propane)/dioleoylphosphatidylethanolamine. Although the investigators might argue that the RAC has previously approved the DMRIE/DOPE system for Drs. Nabel and Rubin's studies, intranasal gene delivery to healthy CF patients raises safety issues different from those posed by intratumoral injection of terminally ill melanoma patients. The investigators have submitted additional data to demonstrate safety of the liposome and expression of a reporter gene in a rat model. These data are still too preliminary to justify approval of the human study. The investigators have outlined several ongoing safety experiments in the rat model involving short-term and long-term toxicity of the DMRIE/DOPE/CFTR construct. The strategy and preliminary data appear reasonable. Therefore, he recommended RAC approval of the protocol contingent on submission of data from these ongoing animal experiments.

Dr. Post asked the investigators to respond to several other questions. Where does the 1 ml volume of the DNA/liposome mixture go after intranasal administration? Would a device designed to prevent nasal drainage be useful? Does the DNA integrate into chromosomes? What is the expected duration of gene expression using this method of delivery? In response to a previous suggestion by Dr. Miller, the investigators have deleted an open reading frame encoding 44 amino acids from the carboxy terminus of the SV40 small T antigen of the pKCTR vector. Has this modification been incorporated into the control vector?

Dr. Walters asked whether the liposome delivery method poses less of a public health concern than adenovirus vector delivery? Dr. Post answered that liposome delivery presents a lesser degree of risk.

Review--Dr. Krogstad

Dr. Krogstad asked about the investigators' degree of confidence, from an anatomic and functional point of view, that the liposome material will remain in the nostril. Could fluorescein be used diagnostically to answer this question? What is the level of sensitivity of the bioelectric potentiometric assay for the detection of CFTR expression? Is this assay sensitive enough to detect differences between the treated and untreated nostrils? Based on Dr. Crystal's results, detection of CFTR expression is not a trivial problem. What are the possible risks associated with liposome delivery of CFTR to the nasal epithelium? Can the knowledge gained from this trial be successfully translated into a therapeutic treatment for CF? From a mechanical point of view, what is the

degree of difficulty that might be encountered when administering this material throughout the tracheobronchial tree?

Review--Dr. Secundy

Dr. Secundy stated the following concerns about the Informed Consent document. This document states that patients should not become pregnant; however, an adequate explanation about the possible risks has not been provided. Due to the duration of this disease, CF patients may be more likely to become pregnant than other terminally ill patients; therefore, pregnancy is an important issue. If a patient becomes pregnant and withdraws from the study, who will provide patient follow-up?

Other Comments

Ms. Grossman commented on the technical problems of measuring the potentiometric differences between the treated nostril and the control nostril. This problem has not been adequately addressed by the investigators. Dr. Parkman asked about the recommended period for contraception since the risk of germ line gene insertion is unknown. Dr. Miller responded that there is minimal risk of germ line integration with local administration; therefore, contraception should not be required. Dr. Miller explained that the 44 amino acid coding sequence of the SV40 small T antigen at the polyadenylation site of the vector construct is unlikely to encode a gene product with transforming activity. Since the plasmid vector is not a virus, there is very little risk of replication and transmission to other individuals.

Investigator Response--Dr. Sorscher

Dr. Sorscher presented a diagram of the vector construct demonstrating that the small region of SV40 T antigen has been removed from the modified DNA construct.

In response to the RAC's concerns about the ability to measure nasal bioelectric potential differences, the proposed techniques yielded consistent measurements. This procedure involves minimal discomfort to the patients. Consistently, 2- to 3-fold differences have been detected between the nostrils of CF patients and normal individuals. Therefore, correction of the CFTR defect should be measurable. Dr. Krogstad suggested that fluorescein could be used as a marker to monitor the amount of material that crosses to the other nostril by ciliary activity.

With regard to the issue of contraception, Dr. Sorscher said the risk of germ line integration is very small since this construct is not a viral vector; however, the risk will be clearly stated in the Informed Consent document. Since the risk is minimal, the contraception requirement will be deleted.

Dr. Miller asked whether inclusion of the ampicillin-resistance gene in the vector construct could compromise the treatment of pneumonia in CF patients? Dr. Sorscher responded that there are many other more useful antibiotics available. Ampicillin is not

the antibiotic of choice for the treatment of pneumonia.

Ms. Meyers recommended that a letter be sent to Dr. Sorcher's IRB requesting that the statement about compensation for research-related injury be deleted from the Informed Consent document.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. Secundy to approve the protocol contingent on the submission of data derived from the ongoing toxicity studies as outlined in Dr. Sorscher's response to Dr. Post dated December 2, 1993. The motion to approve the protocol passed by a vote of 15 in favor, 0 opposed, and 1 abstention.

The RAC recommended that a letter be sent to the University of Alabama's IRB requesting deletion of the following paragraph from the *Special Risks and Discomfort Related to Being Part of a Study of Gene Administration* section of the Informed Consent document:

> "(b) Throughout the study I will continue to be monitored for complications which are normally associated with CF. Should any of these complications develop, I will be given appropriate therapy. If the complications do not appear to be related to the gene transfer protocol, the usual means of payment (for example, insurance) should be arranged. The University of Alabama, Birmingham, and the Children's Hospital of Alabama have made no provision for monetary compensation in the event of physical injury resulting from research and in the event of such injury, medical treatment is provided, but is not provided free of charge."

XX. ADDITION TO APPENDIX D OF THE *NIH GUIDELINES* REGARDING A HUMAN GENE TRANSFER PROTOCOL ENTITLED: *ADENOVIRUS-MEDIATED GENE TRANSFER OF CFTR TO THE NASAL EPITHELIUM AND MAXILLARY SINUS OF PATIENTS WITH CYSTIC FIBROSIS*/DR. WELSH

Review--Dr. Post

Dr. Walters called on Dr. Post to present his primary review on the protocol submitted by Dr. Michael J. Welsh of Howard Hughes Medical Institute, Iowa City, Iowa. Dr. Post stated that this protocol is an extension of Dr. Welsh's first RAC approved protocol in which an adenovirus-CFTR vector was administered to the nasal epithelium of CF patients. This current proposal involves the multiple administration of increasing doses of a new vector to the nasal epithelium as well as the maxillary sinus. Safety of the modified dosing schedule will be demonstrated in the nasal epithelium prior to maxillary sinus administration.

Dr. Post noted that the results obtained from Dr. Welsh's previous trial are promising. Gene transfer for CF is an excellent example of different investigators using separate

approaches that provide informative results. These trials complement each other. The documentation submitted in support of this proposal is superb. Dr. Post commended the investigators for providing a thorough report on the results of the first trial. Data from the cotton rat experiments indicate that large doses of the adenovirus vector can cause inflammation. This *in vivo* data, in addition to Dr. Crystal's report on a possible adverse effect, supports the strategy of characterization of these vectors in the upper respiratory tract prior to lung administration.

Dr. Post stated that the investigators provided excellent responses to the questions presented in his primary review. He asked the investigators to respond to the following additional comments: (1) The investigators propose to use a new adenovirus vector in which a PGK (phosphoglycerate kinase) promoter is used to permit sustained low level CFTR expression. Is there any evidence that the limited duration of CFTR expression with the previous vector is due to promoter shut-off, as opposed to loss the vector DNA? (2) The investigators state that the possibility of recombination of the new types of adenovirus sequences with the 293 vector producer cells is lower than with the new vector than the previous vector. Has the new vector been subjected to the same level of safety testing as the previous vector? (3) In what volume will the vector be administered to the nose versus the maxillary sinus? Have any precautions been introduced to prevent spillage? Dr. Welsh responded that 0.5 ml will be used. The maxillary sinus is self contained; therefore, spillage is not a significant problem. (4) Have preclinical experiments been conducted demonstrating the ability to administer the vector to the maxillary sinus? (5) The investigators have stated that patients with active adenovirus shedding within 3 weeks will be excluded from the study to avoid recombination with wild-type virus. What is the exact period of time indicated by this statement? Dr. Welsh responded that patients demonstrating positive adenovirus cultures 3 weeks prior to the start of the experiment will be excluded from the study. (6) When and how after will biopsies be performed? (7) The subjects will be isolated for 24 hours following vector administration based on the previous observation that virus shedding was absent after this period of time. However, this proposal involves a 200 fold increase in the amount of virus administered. Will this period of isolation be adequate for these increased doses? Dr. Welsh responded that isolation periods greater than 24 hours may be detrimental to CF patients. CF patients must maintain a daily exercise routine. Dr. Post agreed that lack of exercise is a reasonable consideration, but cautioned that the monitoring of virus shedding is an important safety issue. However, it is most likely that the recombinant vector does not pose increased risk over the wild-type virus. (8) What proportion of each vector lot will be assayed for wild-type adenovirus? Dr. Welsh has indicated his intention to request permission to introduce the same promotor used in this vector, PGK, into other adenovirus vectors as outlined in the protocol. These changes would be submitted to the RAC as minor modifications to the current proposal. Dr. Post stated that he would recommend approval of such modifications.

Review--Dr. DeLeon

Dr. DeLeon complimented the investigators on this well-documented protocol. The rationale and schema for the proposal is logical, the end points are clearly defined, and

complete responses to the *Points to Consider* have been provided. The investigators propose to use a modified adenovirus vector construct for which safety and efficacy have been addressed in the preclinical animal studies. Several minor changes should be made to the Informed Consent document. Since this protocol is a Phase I/II study, the term "treatment" should be replaced with the word "procedure," and the term "gene therapy" should be replaced by "gene transfer." Patients should be informed that a determination will be made whether they are seropositive to adenovirus. Since this protocol is well presented and all of her original concerns have been adequately addressed, Dr. DeLeon recommended approval of this proposal.

Review--Mr. Capron (presented by Dr. DeLeon)

Dr. DeLeon summarized Mr. Capron's written review. The protocol was well-presented. Several minor issues concerning the vector construct have been adequately addressed by the investigators. The number of patients to be enrolled on this study is reasonable and will be limited to those individuals who are seropositive to adenovirus. This criterion will facilitate a rapid immune response and minimize risk of virus transmission. Two separate Informed Consent documents have been approved by the IRB, one document for the nasal epithelium study and another document for the maxillary sinus study. Both of these Informed Consent documents are complete, well presented, and understandable to laypersons.

Other Comments

Ms. Grossman said that the investigators have stated that there were several animal deaths in the preclinical studies. Why was the cause of death undetermined? The investigators need to provide an explanation as to why little differences were observed between single and multiple vector administration in the animal studies. Why was mild inflammation observed in animals at multiple high doses of vector? The RAC must decide whether an open ended vector modification should be approved for this study since significant changes in vectors may affect the immunological responses. The investigators should elaborate on their request to decrease the patient isolation period to 24 hours.

Dr. Post suggested that a reasonable compromise regarding the proposed vectors would be for the RAC to approve the use of the previously approved vector (AD2/CFTR-1) as well as the proposed vector (AD2-ORF6/PGK-CFTR). Any future vector modifications should be submitted as requests for minor modifications.

Dr. Leventhal noted that the highest proposed dose, 10^{10} cfu, is a higher dose of adenovirus than the dose that resulted in the adverse effect reported by Dr. Crystal. Dr. Parkman stated that the RAC may want to reconsider the proposed isolation period for the maxillary sinus administration arm of the study since the vector may persist for a longer period in this isolated area. Ms. Meyers stated that the Informed Consent document does not include recommendations for male contraception, period of contraception, long-term follow-up, and responsibility for costs associated with research-

related injury.

Investigator Response--Dr. Welsh

Dr. Welsh responded to the RAC's concerns about the patient isolation period. Patients were isolated for 5 to 6 days in the previous study. This period of time presented several serious problems to the CF patients. Their clinical symptoms were adversely affected by their lack of daily exercise. Patient recruitment was difficult due to the reluctance of these patients to remain in the hospital for almost one week a month while enrolled in the protocol. A prolonged isolation period is unnecessary because these vectors are replication-deficient. In addition, the proposed new vector contains an E4 deletion that limits its survival outside of the patient's body. Despite the extensive use of these vectors in many laboratories, there have been no instances of adverse consequences to laboratory personnel or health care workers. In an unrelated study in which army personnel were inoculated with wild-type adenovirus, the rate of horizontal transmission in these subjects was extremely low. Other investigators have published monkey studies in which a low rate of horizontal transmission was demonstrated. At a previous RAC meeting, Dr. Harold Ginsberg of Columbia University (an *ad hoc* reviewer for the initial CF studies) indicated that the chance of these impaired viruses surviving outside of the clinical setting is extremely low. The IRB has included the requirement that health care workers will be assayed for immunologic responses to these viruses.

Ms. Grossman asked if the proposed vector poses little risk, why will patients be isolated for 24 hours? Dr. Welsh responded that this time period is for the purpose of patient observation. Dr. Miller commented that the half-life of the virus, approximately 2 minutes at 46° C *ex vivo*, is relatively short; therefore, a short isolation period is justified. Dr. Welsh said that complete vector clearance was observed within one day in the previous study. Dr. Miller suggested that the RAC approve the protocol contingent on the stipulation that if virus is detected within 24 hours in a single patient, the isolation period should be extended. Although there are no major safety concerns associated with adenovirus shedding, the RAC should maintain public confidence about gene transfer studies by ensuring the lack of virus shedding. Ms. Grossman stated that although she has provided comments regarding the proposed study, she will abstain from voting on the protocol due to conflict of interest (co-investigator on another RAC-approved CF protocol).

Dr. Welsh said that the unexplained death of some animals in the preclinical studies was unrelated to gene therapy, noting that the control animals developed symptoms. All animals (male and female) were from a single lot of animals. If the new PGK promoter proves to be more efficacious than the previous promoter, a request for a minor modification will be submitted as suggested by Dr. Post.

In response to Ms. Grossman's question about the lack of difference in gene expression between single versus multiple vector administration in cotton rats, Dr. Welsh explained that the context of the experiment was misunderstood. In monkey experiments, the same level of expression of the reporter gene was observed even after 5 administrations of the

vector. The present study is to test toxicity associated with multiple dosing of the vector.

Dr. Welsh responded to the question of vector-induced inflammation. The data derived from animal and human experiments is variable. Variations have been observed between laboratories. Possible explanations for these variations are different vector constructs, different animal species or strains, and the purity of the vector preparations. Dr. Welsh presented data from his murine study in which vector purity affected the outcome of the experiment. At high doses, pure clinical grade vector preparations caused no evidence of inflammation; however, the lesser purity preparations caused peribronchial inflammation. Different animal species appeared to be a variable factor, e.g., the inflammation observed in BALB/c mice was not present in C57/Bl mice.

In regard to Informed Consent document issues, Dr. Welsh stated that contraception will be recommended for both females and males throughout the active phase of the study and for 1 month after this period. Dr. Chase recommended that a letter be sent to Dr. Welsh's IRB about provision of compensation in the event of research-related injury.

Dr. Walters inquired about the degree of discomfort experienced by patients undergoing biopsy of their maxillary sinus. Dr. Scott Graham (an otolaryngologist and co-investigator on this protocol) responded that biopsy of the maxillary sinus is a procedure commonly performed under local anesthesia. A slight degree of discomfort is associated with the procedure; however, the tissue obtained is crucial to understanding pathogenesis if an adverse reaction is encountered.

Committee Motion

A motion was made by Dr. Post and seconded by Dr. DeLeon to approve the protocol with the following stipulations: (1) the investigator retains the option to use either the AD2-ORF6/PGK-CFTR (new) or AD2/CFTR-1 (old) adenovirus vectors, and (2) patients will be isolated for a period of 24 hours following vector administration; however, if a single patient demonstrates virus shedding at 24 hours, the investigator will immediately notify the RAC for reconsideration of the isolation period. The motion to approve the protocol passed by a vote of 11 in favor, 1 opposed, and 3 abstentions.

The RAC recommended that a letter be sent by ORDA to the IRB of the University of Iowa requesting that the following statement be deleted from the Informed Consent document:

> "I understand that in the event of physical injury resulting directly from the research procedures, no compensation will be available in the absence of negligence by a state employee. However, medical treatment is available at the University Hospitals and Clinics, but I will be responsible for making arrangements for payment of the expenses of such treatments..."

XXI. AMENDMENTS TO SECTIONS III, IV, V OF THE *NIH GUIDELINES* AND THE *POINTS TO CONSIDER* REGARDING NIH (ORDA) REVIEW AND APPROVAL OF

CERTAIN CATEGORIES OF HUMAN GENE TRANSFER EXPERIMENTS THAT QUALIFY FOR THE ACCELERATED REVIEW PROCESS/DR. PARKMAN

Dr. Parkman, Chair of the RAC Working Group on Accelerated Review provided a summary of the proposed amendments to the *NIH Guidelines* and *Points to Consider* regarding accelerated review of human gene transfer protocols. The proposed amendments would: (1) establish an accelerated review process for ceratin categories of human gene transfer experiments (i.e., "umbrella" multiple site protocols in which the PI is responsible for quality control and data reporting for research conducted at all sites, duplicate protocols conducted at sites other than those originally approved by the RAC and in which there is a new PI, protocols involving lethally irradiated cells with no replication-competent virus, and modifications to previously approved protocols not related to gene transfer); (2) allow the NIH (ORDA) to assign the appropriate review category to all human gene transfer proposals that are submitted in compliance with *NIH Guidelines*; (3) allow NIH (ORDA) to approve those categories of human gene transfer experiments that qualify for the accelerated review process in consultation with the Chair and one or more RAC members, as necessary; and (4) exempt certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects which are not covered by Footnote 21. All human gene transfer experiments approved by NIH (ORDA) through the accelerated review process would be provided in a report by the Chair at the next regularly scheduled RAC meeting and included in the list of approved experiments which is available form ORDA. Experiments approved through the accelerated review process would be considered *Minor Actions* to the *NIH Guidelines*, eliminating the necessity for full RAC review and publication of the proposed action in the *Federal Register*. Human gene transfer experiments that are not considered as *Minor Actions* or *Exempt* from the *NIH Guidelines* would be considered *Major Actions*, and require publication in the *Federal Register*, full RAC review, and approval by the NIH Director.

Committee Consensus

The consensus of the RAC was that this proposal should be published for public comment in the *Federal Register* and reviewed at the next regularly scheduled RAC meeting.

(Note: The next meeting of the RAC will be March 3-4, 1994, NIH, Building 31C, Conference Room 6.)

XXII. ADJOURNMENT

Dr. Walters adjourned the meeting at 4:45 p.m. on December 3, 1993.

Nelson A. Wivel, M.D.
Executive Secretary

I hereby acknowledge that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Date: 3/3/94

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HUMAN GENE TRANSFER PROTOCOLS

8810-001[a] (M) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2 and Tumor Infiltrating Lymphocytes.*
RAC Approval: 10-3-88/NIH Approval: 3-2-89
Minor Modification: 3-30-90

9007-002[a,b] (T) Blaese, R. Michael, National Institutes of Health, Bethesda, Maryland; *Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells*
RAC Approval: 7-31-90/NIH Approval: 9-6-90
Major Amendment/RAC Approval: 2-10-92
Major Amendment/NIH Approval: 4-22-92
Minor Modification: 4-21-93
Minor Modification: 6-7-93

9007-003 (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Gene Therapy of Patients with Advanced Cancer Using Tumor Infiltrating Lymphocytes Transduced with the Gene Coding for Tumor Necrosis Factor.*
RAC Approval: 7-31-90/NIH Approval: 9-6-90

9102-004[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Hurwitz, Craig; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
RAC Approval: 2-4-91/NIH Approval: 7-12-91
Minor Modification: 11-91

9105-005[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 11-91

9105-006[a] (M) Brenner, Malcolm K.; Mirro, Joseph; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 11-91

9105-007[a] (M) Deisseroth, Albert B., M.D. Anderson Cancer Research Center, Houston, Texas; *Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial.*
RAC Approval: 5-31-91/NIH Approval: 7-12-91
Minor Modification: 4-19-93

9105-008 (M) Ledley, Fred D.; Woo, Savio; Ferry, George; and Hartwell, Whigennand, Baylor College of Medicine, Houston, Texas; *Hepatocellular Transplantation in Acute Hepatic Failure and Targeting Genetic Markers to Hepatic Cells.*
RAC Approval: 5-30-91/NIH Approval: 7-12-91

9105-009[a] (M) Lotze, Michael T., University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; *The Administration of Interleukin-2 and Tumor Infiltrating Lymphocytes to Patients with Melanoma.*
RAC Approval: 5-30-91/NIH Approval: 1-17-92
Minor Modification: 11-30-92

9110-010[a] (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF).*
RAC Approval: 10-7-91/NIH Approval: 10-15-91
Minor Modification: 7-9-92

9110-011 (T) Rosenberg, Steven A., National Institutes of Health, Bethesda, Maryland; *Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Interleukin-2 (IL-2).*
RAC Approval: 10-7-91/NIH Approval: 10-15-91

9110-012[a] (T) Wilson, James M., University of Pennsylvania, Philadelphia, Pennsylvania; *Ex Vivo Gene Therapy of Familial Hypercholesterolemia.*
RAC Approval: 10-8-91/NIH Approval: 11-14-91
Minor Modification: 12-3-92
Minor Modification: 8-18-93

9202-013[a] (T) Nabel, Gary J., University of Michigan, Ann Arbor, Michigan; *Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors.*
RAC Approval: 2-10-92/NIH Approval: 4-17-92
Minor Modification: 1-22-93
Minor Modification: 1-93

9202-014 (M) Cornetta, Kenneth, Indiana University, Indianapolis, Indiana; *Retroviral-Mediated Gene Transfer of Bone Marrow Cells during Autologous Bone Marrow Transplantation for Acute Leukemia.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92

9202-015 (M) Economou, James S. and Belldegrun, Arie, University of California at Los Angeles, Los Angeles, California; *The Treatment of Patients with Metastatic Melanoma and Renal Cell Cancer Using In Vitro Expanded and Genetically-Engineered (Neomycin Phosphotransferase) Bulk, CD8 (+) and/or CD4(+) Tumor Infiltrating Lymphocytes and Bulk, CD8(+) and/or CD4(+) Peripheral Blood Leukocytes in Combination with Recombinant Interleukin-2 Alone, or with Recombinant Interleukin-2 and Recombinant Alpha Interferon.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92

9202-016[a] (T) Freeman, Scott M., Tulane University Medical Center, New Orleans, Louisiana; *Gene Transfer for the Treatment of Cancer.*
RAC Approval: 2-10-92/NIH Approval: 2-5-93
Minor Modification: 8-6-93

9202-017[b] (M) Greenberg, Philip D. and Riddell, Stanley, Fred Hutchinson Cancer Research Center, University of Washington, Seattle; *Phase I Study to Evaluate the Safety of Cellular Adoptive Immunotherapy using Genetically Modified CD8+ HIV-Specific T Cells in HIV Seropositive Individuals.*
RAC Approval: 2-11-92/NIH Approval: 4-17-92
Major Amendment/RAC Approval: 9-9-93
Major Amendment/NIH Approval: 10-7-93

9206-018 (T) Brenner, Malcolm K.; Furman, Wayne; Santana, Victor; Bowman, Laura; and Meyer, William, St. Jude Children's Research Hospital, Memphis, Tennessee; *Phase I Study of Cytokine-Gene Modified Autologous Neuroblastoma Cells for Treatment of Relapsed/Refractory Neuroblastoma.*
RAC Approval: 6-1-92/NIH Approval: 8-14-92

9206-019 (T) Oldfield, Edward, National Institutes of Health, Bethesda, Maryland; *Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir.*
RAC Approval: 6-1-92/NIH Approval: 8-14-92
Minor Modification: 11-29-93

9206-020[a] (M) Deisseroth, Albert B., MD Anderson Cancer Center, Houston, Texas; *Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 4-19-93

9206-021 (T) Gansbacher, Bernd; Houghton, Alan; and Livingston, Philip, Memorial Sloan Kettering Cancer Center, New York, New York; *Immunization with HLA-A2 matched Allogeneic Melanoma Cells that Secrete Interleukin-2 in Patients with Metastatic Melanoma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92

9206-022[a] (T) Gansbacher, Bernd; Motzer, Robert; Houghton, Alan; and Bander, Neil, Memorial Sloan Kettering Cancer Center, New York, New York; *Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92
Minor Modification: 3-31-93

9206-023[a] (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Multiple Myeloma.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92

9206-024[a] (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Metastatic Breast Cancer.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92

9206-025[a] (M) Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland; *Retroviral-Mediated Gene Transfer of Bone Marrow and Peripheral Blood Stem Cells During Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia.*
RAC Approval: 6-2-92/NIH Approval: 8-14-92

9209-026 (M) Walker, Robert E., National Institutes of Health, Bethesda, Maryland; *A Study of the Safety and Survival of the Adoptive Transfer of Genetically Marked Syngeneic Lymphocytes in HIV Infected Identical Twins.*
RAC Approval: 9-14-92/NIH Approval: 9-3-93

9209-027 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *A Phase I/II Study of the Use of Recombinant Human Interleukin 3 (rhil3) Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Breast Carcinoma or Hodgkin's Disease.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93

9209-028 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *Evaluation of the Use of Recombinant Human G-CSF Stimulated Peripheral Blood Progenitor Cell Supplementation in Autologous Bone Marrow Transplantation in Patients with Lymphoid Malignancies.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93

9209-029 (M) Schuening, Friedrich G., Fred Hutchinson Cancer Research Center, University of Washington, Seattle, Washington; *A Trial of G-CSF Stimulated Peripheral Blood Stem Cells for Engraftment in Identical Twins.*
RAC Approval: 9-14-92/NIH Approval: 2-5-93

9209-030 (M) Deisseroth, Albert B., University of Texas MD Anderson Cancer Center, Houston, Texas; *Use of Retroviral Markers to Identify Efficacy of Purging and Origin of Relapse Following Autologous Bone Marrow and Peripheral Blood Cell Transplantation in Indolent B Cell Neoplasms (Follicular Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia) Patients.*
RAC Approval: 9-14-92
Minor Modification: 11-3-93
Minor Modification: 11-18-93

9209-031 (T) Roth, Jack A., The University of Texas MD Anderson Cancer Center, Houston, Texas; *Clinical Protocol for Modification of Oncogene and Tumor Suppressor Gene Expression in Non-Small Cell Lung Cancer.*
RAC Approval: 9-15-92

9209-032 (M) Brenner, Malcolm K., St. Jude Children's Research Hospital, Memphis, Tennessee; *A Phase II Trial of the Baxter Neuroblastoma Bone Marrow Purging System Using Gene Marking to Assess Efficacy.*
RAC Approval: 9-15-92/NIH Approval: 2-5-93

9209-033 (T) Lotze, Michael T. and Rubin, Joshua T., University of Pittsburgh, Pittsburgh, Pennsylvania; *Gene Therapy of Cancer: A Pilot Study of IL-4 Gene Modified Antitumor Vaccines.*
RAC Approval: 9-15-92/NIH Approval: 2-5-93

9212-034[a] (T) Crystal, Ronald G., National Institutes of Health, Bethesda, Maryland; *A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.*
RAC Approval: 12-3-92/NIH Approval: 4-16-93
Minor Modification: 5-17-93
Minor Modification: 10-8-93
Minor Modification: 11-29-93

9212-035[a] (T) Wilson, James M., University of Pennsylvania, Philadelphia, Pennsylvania; *Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial.*
RAC Approval: 12-3-92/NIH Approval: 8-26-93
Minor Modification: 8-17-93

9212-036 (M) Welsh, Michael J., Howard Hughes Medical Institute, Iowa City, Iowa, and Smith, Alan E., Genzyme Corporation, Framingham, Massachusetts; *Cystic Fibrosis Gene Therapy Using an Adenovirus Vector: In Vivo Safety and Efficacy in Nasal Epithelium.*
RAC Approval: 12-4-92/NIH Approval: 4-16-93
Minor Modification: 10-18-93

9303-037 (T) Culver, Kenneth W., Iowa Methodist Medical Center, Des Moines, Iowa, and Van Gilder, John C.; University of Iowa, Iowa City, Iowa; *Gene Therapy for the Treatment of Malignant Brain Tumors with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene/Ganciclovir System.*
RAC Approval: 3-1-93/NIH Approval: 4-16-93

9303-038 (M) Heslop, Helen E.; Brenner, Malcolm K.; and Rooney, Cliona, St. Jude Children's Research Hospital, Memphis, Tennessee; *Administration of Neomycin Resistance Gene Marked EBV Specific Cytotoxic T Lymphocytes to Recipients of Mismatched-Related or Phenotypically Similar Unrelated Donor Marrow Grafts.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-039 (M) Brenner, Malcolm K.; Krance, Robert; Heslop, Helen E.; Santana, Victor; and Ihle, James, St. Jude Children's Research Hospital, Memphis, Tennessee; *Assessment of the Efficacy of Purging by Using Gene-Marked Autologous Marrow Transplantation for Children with Acute Myelogenous Leukemia in First Complete Remission.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-040 (T) Simons, Jonathan, Johns Hopkins Oncology Center, Baltimore, Maryland; *Phase I Study of Non-Replicating Autologous Tumor Cell Injections Using Cells Prepared With or Without Granulocyte-Macrophage Colony Stimulating Factor Gene Transduction in Patients with Metastatic Renal Cell Carcinoma.*
RAC Approval: 3-1-93

9303-041 (T) Wilmott, Robert W. and Whitsett, Jeffrey, Children's Hospital Medical Center, Cincinnati, Ohio, and Trapnell, Bruce; Genetic Therapy, Inc., Gaithersburg, Maryland; *A Phase I Study of Gene Therapy of Cystic Fibrosis Utilizing a Replication Deficient Recombinant Adenovirus Vector to Deliver the Human Cystic Fibrosis Transmembrane Conductance Regulator cDNA to the Airways.*
RAC Approval: 3-2-93/NIH Approval: 4-16-93

9303-042 (T) Boucher, Richard C. and Knowles, Michael R., University of North Carolina, Chapel Hill, North Carolina; *Gene Therapy for Cystic Fibrosis Using E1 Deleted Adenovirus: A Phase I Trial in the Nasal Cavity.*
RAC Approval: 3-2-93/NIH Approval: 10-7-93

9306-043 (T) Seigler, Hilliard F., Duke University Medical Center, Durham, North Carolina; *A Phase I Trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients With Disseminated Malignant Melanoma.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-044 (T) Deisseroth, Albert B.; Kavanagh, John; and Champlin, Richard, University of Texas MD Anderson Cancer Center, Houston, Texas; *Use of Safety-Modified Retroviruses to Introduce Chemotherapy Resistance Sequences into Normal Hematopoietic Cells for Chemoprotection During the Therapy of Ovarian Cancer: A Pilot Trial.*
RAC Approval: 6-7-93

9306-045 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *Immunotherapy for Cancer by Direct Gene Transfer into Tumors.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-046 (T) Barranger, John A., University of Pittsburgh, Pittsburgh, Pennsylvania, *Gene Therapy for Gaucher Disease: Ex Vivo Gene Transfer and Autologous Transplantation of CD34(+) Cells.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-047 (T) Karlsson, Stefan and Dunbar, Cynthia, National Institutes of Health, Bethesda, Maryland, and Kohn, Donald B., Childrens Hospital Los Angeles, Los Angeles, California; *Retroviral Mediated Transfer of the cDNA for Human Glucocerebrosidase into Hematopoietic Stem Cells of Patients with Gaucher Disease.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-048 (T) Galpin, Jeffrey E., University of Southern California, and Casciato, Dennis A., University of California, Los Angeles, California; *A Preliminary Study to Evaluate the Safety and Biologic Effects of Murine Retroviral Vector Encoding HIV-1 Genes [HIV-IT(V)] in Asymptomatic Subjects Infected with HIV-1.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-049 (T) Nabel, Gary J., University of Michigan Medical Center, Ann Arbor, Michigan; *A Molecular Genetic Intervention for AIDS - Effects of a Transdominant Negative Form of Rev.*
RAC Approval: 6-7-93/NIH Approval: 9-3-93

9306-EXP (T) Sobol, Robert and Royston, Ivor, San Diego Regional Cancer Center, San Diego, California; *CONFIDENTIAL.*
NIH Approval: 12-28-92
Major Amendment (Expedited Review)/NIH Approval: 5-11-93

9306-050 (T) Raffel, Corey, Childrens Hospital Los Angeles, Los Angeles, California, and Culver, Kenneth, Iowa Methodist Medical Center, Des Moines, Iowa; *Gene Therapy for the Treatment of Recurrent Pediatric Malignant Astrocytomas with In Vivo Tumor Transduction with the Herpes Simplex Thymidine Kinase Gene.*
RAC Approval: 6-8-93/NIH Approval: 9-3-93

9306-051 (T) Hesdorffer, Charles and Antman, Karen, Columbia University College of Physicians and Surgeons, New York, New York; *Human MDR Gene Transfer in Patients with Advanced Cancer.*
RAC Approval: 6-8-93/NIH Approval: 9-3-93

9306-052 (T) Ilan, Joseph, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio; *Gene Therapy for Human Brain Tumors Using Episome-Based Antisense cDNA Transcription of Insulin-Like Growth Factor I.*
RAC Approval: 6-8-93

9309-053 (T) Cassileth, Peter; Podack, Eckhard R.; Sridhar, Kasi; University of Miami, and Savaraj, Niramol; Miami Veterans Administration Hospital, Miami, Florida; *Phase I Study of Transfected Cancer Cells Expressing the Interleukin-2 Gene Product in Limited Stage Small Cell Lung Cancer.*
RAC Approval: 9-9-93

9309-054 (T) O'Shaughnessy, Joyce; National Institutes of Health, Bethesda, Maryland; *Retroviral Mediated Transfer of the Human Multi-Drug Resistance Gene (MDR-1) into Hematopoietic Stem Cells During Autologous Transplantation after Intensive Chemotherapy for Breast Cancer.*
RAC Approval: 9-9-93

9309-055 (T) Kun, Larry E.; Sanford, R. A.; Brenner, Malcolm K.; and Heideman, Richard L.; St. Jude Childrens Research Hospital, Memphis, Tennessee; and Oldfield, Edward H.; National Institutes of Health, Bethesda, Maryland; *Gene Therapy for Recurrent Pediatric Brain Tumors.*
RAC Approval: 9-9-93

9309-056 (T) Das Gupta, Tapas K. and Cohen, Edward P.; University of Illinois at Chicago; Chicago, Illinois; *Immunization of Malignant Melanoma Patients with Interleukin 2-Secreting Melanoma Cells Expressing Defined Allogeneic Histocompatibility Antigens.*
RAC Approval: 9-10-93

9309-057 (T) Wong-Staal, Flossie; Poeschla, Eric; and Looney, David; University of California, San Diego, California; *A Phase I Clinical Trial to Evaluate the Safety and Effects in HIV-1 Infected Humans of Autologous Lymphocytes Transduced with a Ribozyme that Cleaves HIV-1 RNA.*
RAC Approval: 9-10-93

9309-058 (T) Economou, James S. and Glasby, John A.; University of California Medical Center, Los Angeles, California; *Genetically Engineered Autologous Tumor Vaccines Producing Interleukin-2 for the Treatment of Metastatic Melanoma.*
RAC Approval: 9-10-93

	T	M	Total (T + M)
RAC APPROVED	35	23	58[c]
NIH DIRECTOR APPROVED	28	22	50

LEGEND:

M = Human Gene Marking Protocol
T = Human Gene Therapy Protocol
[a] = Minor Modification(s)
[b] = Major Amendment(s)
[c] = The number of protocols appearing in the table is greater than the number that appears in the protocol list because of the single *Expedited Review* protocol that was approved by the NIH Director on 12-28-92.
_ = RAC Approved/NIH Approval Pending

January 31, 1994

MINOR MODIFICATIONS TO NIH-APPROVED HUMAN GENE TRANSFER PROTOCOLS

DATE OF MINOR MODIFI-CATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
3/30/90	3/2/89	8810-001	Rosenberg, Steven A.	The Treatment of Patients with Advanced Cancer Using Cyclophosphamide, Interleukin-2, and Tumor Infiltrating Lymphocytes	Lift the cap on the number of patients accrued onto the protocol.
11/91	7/12/91	9102-004	Brenner, Malcolm K., et. al.	Autologous Bone Marrow Transplant for Children with Acute Myelogenous Leukemia in First Complete Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-005	Brenner, Malcolm K., et. al.	A Phase I/II Trial of High Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Stage D Neuroblastoma in First Remission: Use of Marker Genes to Investigate the Biology of Marrow Reconstitution and the Mechanism of Relapse	Use the retrovirus vector, G1Na, instead of LNL6.
11/91	7/12/91	9102-006	Brenner, Malcolm K., et. al.	A Phase II Trial of High-Dose Carboplatin and Etoposide with Autologous Marrow Support for Treatment of Relapse/Refractory Neuroblastoma Without Apparent Bone Marrow Involvement	Use the retrovirus vector, G1Na, instead of LNL6.
7/9/92	10/15/91	9110-010	Rosenberg, Steven A.	Immunization of Cancer Patients Using Autologous Cancer Cells Modified by Insertion of the Gene for Tumor Necrosis Factor (TNF)	Extend patient eligibility criteria to include patients with advanced breast cancer.
11/30/92	1/17/92	9105-009	Lotze, Michael T.	The Administration of Interleukin-2, ~~Interleukin-4,~~ and Tumor Infiltrating Lymphocytes to Patients with Melanoma	(1) Eliminate systemic IL-4 therapy, and (2) allow administration of cyclophosphamide prior to TIL infusions.
12/3/92	11/14/91	9110-012	Wilson, James M.	Ex Vivo Gene Therapy of Familial Hypercholesterolemia	Increase patient accrual from 3 to 5 patients.
1/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	(1) addition of assays for anti-DNA antibodies on day 28 and week 8; (2) repeat chest and abdominal CTs, when indicated, at 6-8 weeks following initial treatment; (3) additional biopsies performed on days 16 and 20 and as needed to verify gene expression; (4) reduce the duration of the protocol from 16 to 8 weeks; and (5) expand patient eligibility to include those patients who have tolerated previous treatments without side effects at higher doses.

DATE OF MINOR MODIFI-CATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
1/22/93	4/17/92	9202-013	Nabel, Gary J.	Immunotherapy of Malignancy by In Vivo Gene Transfer into Tumors	Deliver HLA-B7 DNA liposome complexes by catheter to pulmonary nodules.
3/31/93	8/14/92	9206-022	Gansbacher, Bernd, et. al.	Immunization with Interleukin-2 Secreting Allogeneic HLA-A2 Matched Renal Cell Carcinoma Cells in Patients with Advanced Renal Cell Carcinoma	Use only irradiated SK-RC-39 cell line, not SK-RC-28.
4/19/93	7/12/91	9105-007	Deisseroth, Albert B.	Autologous Bone Marrow Transplantation for Chronic Myelogenous Leukemia in which Retroviral Markers are Used to Discriminate between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Leukemic Cells in Autologous Marrow: A Pilot Trial	New transduction protocol which allows the use of autologous stroma and growth factors.
4/19/93	8/14/92	9206-020	Deisseroth, Albert B.	Use of Two Retroviral Markers to Test Relative Contribution of Marrow and Peripheral Blood Autologous Cells to Recovery After Preparative Therapy	Revised transduction procedure that includes the use of autologous stroma and growth factors.
4/21/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells	Treat newborns with ADA deficiency with ADA gene-corrected autologous hematopoietic stem cells obtained from the patients' umbilical cord and placenta.
5/17/93	4/16/93	9212-034	Crystal, Ronald G.	A Phase I Study, in Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient. Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung.	Decrease the "baseline" and "vehicle control" portions of the protocol from 1 month to 1 week each.
6/7/93	9/6/90 Major Amendment 4/22/92	9007-002	Blaese, R. Michael	Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells	The investigator is permitted to: (1) withdraw PEG-ADA at his discretion, and (2) perform bone marrow aspirations as necessary to monitor for gene transduction.
8/6/93	2/5/93	9202-016	Freeman, Scott M.	Gene Transfer for the Treatment of Cancer	PI has moved to Tulane University Medical Center. The total number of patients will be divided between Tulane University and the University of Rochester.

DATE OF MINOR MODIFICATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
8/18/93	8/25/93	9212-035	Wilson, James M.	Gene Therapy of Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.
8/19/93	11/14/91	9110-012	Wilson, James M.	Ex Vivo Gene Therapy for Familial Hypercholesterolemia	PI has moved to the University of Pennsylvania Medical Center. Patients will not be treated at the University of Michigan.
8/26/93	8/26/93	9212-035	Wilson, James M.	Gene Therapy for Cystic Fibrosis Lung Diseases Using E1 Deleted Adenoviruses: A Phase I Trial	(1) the lowest dose of adenovirus vector will be decreased by 100-fold, (2) the highest dose will be decreased by 10-fold, (3) the incremental dose between the patient groups will be reduced from 10-fold to 3-fold, (4) the number of patients within each group will be reduced from three to two, and (5) the total number of patients enrolled will be increased from 12 to 20.
10/8/93	4/16/93	9212-034	Crystal, Ronald, G.	Phase I Study, In Cystic Fibrosis Patients, of the Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung	(1) Change in dosing schedule, (2) increase time interval between nasal and lung administration from 1 to 2 days, (3) the time interval between patients within each dosing group will be changed to 2 weeks, (4) the time interval between the dose groups will be changed to 3 weeks, (5) isolation period will be reduced from 10 to 2 days, (6) eliminate requirement for negative pressure isolation of patients, (7) inpatient requirement will be reduced from 2 months to 2 weeks (continent on 3 consecutive negative cultures), (8) addition of bronchial biopsies, (9) elimination of scintigraphic assessment, (10) elimination of quantitation of sputum volume and sputum culture, (11) patients will enter the 7 day vehicle control period within 2 months of completion of the 7 day baseline period and will receive vector administration within 2 months of completion of the vehicle control period, and (12) the number of chest CT scans will be reduced to 1 baseline scan unless indicated for clinical purposes. Contingency: any patients demonstrating unexpected side effects will receive a CT scan.

DATE OF MINOR MODIFI-CATION	DATE OF NIH APPROVAL	PROTOCOL #	PRINCIPAL INVESTIGATOR(S)	PROTOCOL TITLE	MINOR MODIFICATION
10/18/93	4/16/93	9212-036	Welsh, Michael J.	Cystic Fibrosis Gene Therapy Using an Adenovirus Vector: In Vivo Safety and Efficacy in Nasal Epithelium	(1) add an additional 3 patients; (2) each additional patient will receive $5x10^7$ IU of the adenovirus vector; (3) the adenovirus vector will be applied to only 1 nostril (saline in the other as a control); (4) the virus treated and saline control tissues will be biopsied (patient #1-day 1, patient #2-day 3, and patient #3-day 7); (5) use an alternative method of vector delivery (small catheter); (6) delete adenosine administration, (7) replace skin patch with subcutaneous needle for nasal epithelium voltage measurements; (8) decrease the period of hospitalization from 2-3 weeks to 5-7 days; and (9) eliminate nasal brushings.
11/3/93	Pending	9209-030	Deisseroth, Albert B.	Use of Retroviral Markers to Evaluate the Efficacy of Purging and to Discriminate Between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Neoplastic Cells in Autologous Marrow Following Purging in Patients with Chronic Lymphocytic Leukemia (CLL)	(1) Addition of PIXY321 growth factor during retrovirus transduction, and (2) perform stromal monolayers 2 weeks before retrovirus transduction.
11/18/93	Pending	9209-030	Deisseroth, Albert B.	Use of Retroviral Markers to Evaluate the Efficacy of Purging and to Discriminate Between Relapse which Arises from Systemic Disease Remaining after Preparative Therapy Versus Relapse due to Residual Neoplastic Cells in Autologous Marrow Following Purging in Patients with Chronic Lymphocytic Leukemia (CLL)	Expand patient eligibility criteria to include indolent non-Hodgkin's lymphoma (NHL) patients. The revised protocol title reads: Use of Retroviral Markers to Identify Efficacy of Purging and Origin of Relapse Following Autologous Bone Marrow and Peripheral Blood Cell Transplantation in Indolent B Cell Neoplasms (Follicular Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia) Patients
11/29/93	4/16/93	9212-034	Crystal, Ronald G.	Phase I Study, in Cystic Fibrosis Patients, of Safety, Toxicity, and Biological Efficacy of a Single Administration of a Replication Deficient, Recombinant Adenovirus Carrying the cDNA of the Normal Human Cystic Fibrosis Transmembrane Conductance Regulator Gene in the Lung	(1) Expand the CFTR genotype eligibility criteria from the delta F508 genotype to "any CFTR genotype", and (2) delete the 3-day waiting period between the baseline and vehicle control periods and between the vehicle control and experimental treatment periods.
11/29/93	8/14/92	9206-019	Oldfield, Edward H.	Gene Therapy for the Treatment of Brain Tumors Using Intra-Tumoral Transduction with the Thymidine Kinase Gene and Intravenous Ganciclovir	The number of patients accrued onto the study will remain the same; however, the numerical constraints as to each diagnostic group (primary versus metastases) will be eliminated.

RECOMBINANT DNA ADVISORY COMMITTEE (RAC)
DATA MANAGEMENT REPORT - DECEMBER 1993*

Protocol #	PI(s)	Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector/ Gene	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patient Accrual To Date	In Vivo Evidence of Gene Transfer (Human)	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
8810-001[a]	Rosenberg	NIH	10-3-88	3-2-89	M	Cancer	5-22-89	LNL6/Neo^R	PA317	GTI	Unlimited	10	Yes	No	No	9	Open
9007-002[a,b]	Blaese	NIH	7-31-90 2-10-92[b]	9-6-90 4-22-92[b]	T	SCID (ADA-defic.)	9-14-90 5-13-93	LASN-ADA/ Neo^R G1NaSvAd/ Neo^R	PA317 PA317	GTI GTI	10	2-PBL 1-PBSC 3-CBSC	Yes	No	No	0	Open
9007-003	Rosenberg	NIH	7-31-90	9-6-90	T	Cancer	1-29-91	TNF-Neo^R	PA317	GTI/ Chiron	50	12	Yes	No	1 - transient elevation of liver function	8	Open
9102-004[a]	Brenner, et. al.	St. Jude	2-4-91	7-12-91	M	BMM/ AML	9-9-91	G1Na/Neo^R	PA317	GTI	12	12	Yes	No	No	4	Closed
9105-005[a]	Brenner, et. al.	St. Jude	5-31-91	7-12-91	M	BMM/ NBL	1-27-92	G1Na/Neo^R	PA317	GTI	10	6	Yes	No	No	0	Closed
9105-006[a]	Brenner, et. al.	St. Jude	5-31-91	7-12-91	M	Cancer/ NBL	1-16-92	G1Na/Neo^R	PA317	GTI	10	3	Yes	No	No	1	Closed
9105-007[a]	Deisseroth	MD Anderson	5-31-91	7-12-91	M	BMM/ CML	7-31-92	LNL6/Neo^R	PA317	GTI	10	10 entered/ 5 transplanted	Yes	No	ND-1 patient failed to engraft	7--3 transplanted	Closed
9105-008	Ledley, et. al.	Baylor	5-30-91	7-12-91	M	Acute Hepatic Failure	P	LNL6/Neo^R	PA317	GTI	6	0	P	P	P	P	Open
9105-009[a]	Lotze	U. of Pittsburgh	5-30-91	1-17-92	M	Cancer/ Melanoma	3-3-92	LNL6/Neo^R	PA317	GTI	20	4	Yes	No	No	1	Open
9110-010[a]	Rosenberg	NIH	10-7-91	10-15-91	T	Cancer	10-8-91	TNF-Neo^R	PA317	GTI/ Chiron	5	3	Yes	No	No	3	Open
9110-011	Rosenberg	NIH	10-7-91	10-15-91	T	Cancer	3-12-92	G1NaCVi2	PA317	GTI	5	2	Yes	No	No	2	Open
9110-012[a]	Wilson	U. of PA	10-8-91	11-14-91	T	FH	6-5-92	CMV-BA-(h)LDLR	Ψ-crip	U. of PA	5	3	Yes	No	No	0	Open

Protocol #	PI(s)	Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector/ Gene	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patient Accrual To Date	In Vivo Evidence of Gene Transfer (Human)	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9202-013[a]	Nabel	U. of MI	2-10-92	4-17-92	T	Cancer	6-3-92	Cationic liposomes/ HLA-B7 cDNA	NA	Vical	12	5	Yes	No	No	0	Closed
9202-014	Cornetta	Indiana U.	2-11-92	4-17-92	M	BMM/ AML ALL	5-15-92	G1Na/Neo^R	PA317	GTI	20 (10 AML and 10 ALL)	4 AML 1 ALL	Yes	Bo	No	1	Open
9202-015	Economou	UCLA	2-11-92	4-17-92	M	Cancer/ Melanoma Renal Cell Cancer	2-19-93	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	40 (20 Melanoma and 20 Renal Cell)	6	Yes	No	No	0	P
9202-016	Freeman	Tulane U.	2-10-92	2-5-93	T	Cancer/ Ovarian	8-93	HSV-TK	PA317	U. of Rochester	20	1	No	No	No	0	Open
9202-017[b]	Greenberg, et. al.	Fred Hutchinson	2-11-92 9-9-93[b]	4-17-92 10-7-93[b]	M	HIV(+)	2-26-93	HyTK	PA317	TGC	30 (15 NHL/ HIV+ and 15 HIV+)	1	Yes	No	No	1-GVH	Open
9206-018	Brenner, et. al.	St. Jude	6-1-92	8-14-92	T	Cancer/ NBL	12-9-92	G1NaCvi2	PA317	GTI	12	3	No	No	No	1	Open
9206-019	Oldfield	NIH	6-1-92	8-14-92	T	Cancer/ Brain	12-7-92	G1TkSvNa	PA317	GTI	20 (10 primary, 10 mets.)	10 (9 primary, 1 met.)	No	ND (hemorrhage/unexpected toxicity)	No	4(3 primary, 1 met.)	Open
9206-020[a]	Deisseroth	MD Anderson	6-2-92	8-14-92	M	BMM/ CML	P	G1Na/Neo^R	PA317	GTI	10	0	P	P	P	P	Open
9206-021	Gansbacher, et. al.	MSKCC	6-2-92	8-14-92	T	Cancer/ Melanoma	2-17-93	NAPAD/IL2	GP+env AM12	MSKCC	12	10	No	No	No	4	Open
9206-022[a]	Gansbacher, et. al.	MSKCC	6-2-92	8-14-92	T	Cancer/ Renal Cell	3-17-93	NAPAD/IL2	GP+env AM12	MSKCC	12	6	No	No	No	2	Open
9206-023	Dunbar	NIH	6-2-92	8-14-92	M	BMM/ Multiple Myeloma	3-18-93	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	6	Yes	No	No	0	Open

Protocol #	PI(s)	Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector/ Gene	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patient Accrual To Date	In Vivo Evidence of Gene Transfer (Human)	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9206-024	Dunbar	NIH	6-2-92	8-14-92	M	BMM/ Breast Cancer	12-7-92	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	5	Yes	No	No	1	Open
9206-025	Dunbar	NIH	6-2-92	8-14-92	M	BMM/ CML	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	0	P	P	P	P	P
9206-026	Walker	NIH	9-14-92	9-3-93	M	HIV+/ Identical Twin	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	0	P	P	P	P	P/FDA
9209-027	Schuening	Fred Hutchinson	9-14-92	2-5-93	M	BMM/ Breast Hodgkin's Disease	P	LN/neo^R	PA317	TGC	20	0	P	P	P	P	Closed
9209-028	Schuening	Fred Hutchinson	9-14-92	2-5-93	M	BMM/ Lymphoid Malig.	P	LN/neo^R	PA317	TGC	20	0	P	P	P	P	Closed
9209-029	Schuening	Fred Hutchinson	9-14-92	2-5-93	M	BMM/ Identical Twins	P	LN/neo^R	PA317	TGC	20	0	P	P	P	P	P/FDA
9209-030	Deisseroth	MD Anderson	9-14-92	P	M	BMM/ CLL	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	10	0	P	P	P	P	P/FDA and NIH
9209-031	Roth	MD Anderson	9-15-92	P	T	Cancer/ NSCLC	P	LNSX-AS-K-ras and LNSX-p53	GP+env AM12	GTI	14	0	P	P	P	P	P/FDA and NIH
9209-032	Brenner	St. Jude	9-15-92	2-5-93	M	BMM/ NBL	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	12	0	P	P	P	P	P/FDA
9209-033	Lotze	U. of Pittsburgh	9-15-92	2-5-93	T	Cancer	9-9-93	G1ILASvNa	PA317	GTI	20	1	P	P	P	0	Open
9212-034[a]	Crystal	Cornell	12-3-92	4-16-93	T	CF	4-17-93	AdCFTR (type 5)	293	Cornell	12	4	Yes	No	Yes (transient, mild, inflammation)	0	Open

Protocol #	PI(s)	Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector/ Gene	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patient Accrual To Date	In Vivo Evidence of Gene Transfer (Human)	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9212-035[a]	Wilson	U. of PA	12-3-92	8-26-93	T	CF	9-3-93	AD.CB-CFTR (type 5)	293	U. of PA	20	2	P	P	P	0	Open
9212-036[a]	Welsh, et. al	HHMI	12-4-92	4-16-93	M	CF	7-26-93	Ad2/CFTR-1 (type 2)	293	Genzyme	P	3	Yes	No	No	0	P/FDA for additional 3
9303-037	Culver, et. al.	Iowa Methodist and U. of IA	3-1-93	4-16-93	T	Cancer/ Brain	P	G1TKSvNa	PA317	GTI	15	0	P	P	P	P	Open
9303-038	Heslop	St. Jude	3-2-93	4-16-93	M	BMM/ Leukemia	P	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	35	0	P	P	P	P	P/FDA
9303-039	Brenner, et. al	St. Jude	3-2-93	4-16-93	M	BMM/ AML	10/12/93	LNL6/Neo^R and G1Na/Neo^R	PA317	GTI	35	2	P	P	P	P	Open
9303-040	Simons	Johns Hopkins	3-1-93	P	T	Cancer/ Renal Cell	P	MFG-hu-GM-CSF			26	0	P	P	P	P	P/FDA and NIH
9303-041	Wilmott, et. al.	Children's Hospital, OH	3-2-93	4-16-93	T	CF	P	Av1CF2 (type 5)	293	GTI	15	0	P	P	P	P	P/FDA
9303-042	Boucher, et. al.	U. of NC	3-2-93	10-7-93	T	CF	P	Ad.CB-CFTR (type 5)	293	U. of MI	9	0	P	P	P	P	Open
9306-043	Seigler	Duke U.	6-7-93	9-3-93	T	Cancer/ Melanoma	8-10-93	pHUγ-IFN	VCHUγ	Viagene	20	0	No	No	No	No	Open
9306-044	Deisseroth, et. al.	MD Anderson	6-7-93	P	T	Cancer/ Ovarian	P	MDR-1 cDNA/LN	PA317	ATCC	20	0	P	P	P	P	P/FDA and NIH
9306-045	Nabel	U. of MI	6-7-93	9-3-93	T	Cancer	P	Cationic liposome (pHLA-B7/ β-2 μglobulin)	NA	Vical	24 (12 intratumor and 12 catheter)	0	P	P	P	P	P/FDA
9306-046	Barranger	U. of Pittsburgh	6-7-93	9-3-93	T	Gaucher Disease	P	R-GC	Ψ-crip	U. of Pittsburgh	5	P	P	P	P	P	P/FDA

Protocol #	PI(s)	Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector/ Gene	Packag-ing Cell	Vector Supplier	Maximum Patient Accrual	Patient Accrual To Date	In Vivo Evidence of Gene Transfer (Human)	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9306-047	Karlsson, et. al	NIH	6-7-93	9-3-93	T	Gaucher Disease	P	G1Gc	PA317	GTI	10 (5-ABM 5-PBL)	0	P	P	P	P	P/FDA
9306-048	Galpin, et. al.	USC and UCLA	6-7-93	9-3-93	T	HIV(+)	8-10-93	[HIV-IT(V)]	D-17	Viagene	15 (3 groups of 5)	12	No	No	No	0	Open
9306-049	Nabel	U. of MI	6-7-93	9-3-93	T	HIV(+)	P	(Rev M10) (ΔRev M10) and (RSV-tar/ Rev M10) (RSV-tar/ Rev M10)	Ψ-crip	Vical	12 (4 groups of 3)	0	P	P	P	P	P/FDA
9306-EXP[c]	Sobol, et. al.	SDRCC	N/A	12-28-93 5-11-93[c]	T	Cancer/ glio-blastoma	1-4-93	12-28-93 (NAPAD-IL2) 5-11-93 (G1NaCv2)	GP+env AM12 PA317	MSKCC GTI	1	1	No	No	No	1	Closed
9306-050	Raffel, et. al.	Children's Hosp., LA and IA Methodist	6-8-93	9-3-93	T	Cancer/ Astro-cytoma	P	G1TkSvNa	PA317	GTI	15 (if + response-additional 15 may be accrued)	0	P	P	P	P	Open
9306-051	Hesdorffer, et. al.	Columbia U.	6-8-93	9-3-93	T	Cancer/ Breast, Ovarian, and Brain	P	pHAMDR1/ A	GP+env AM12	MA and GTI	20	0	P	P	P	P	P/FDA
9306-052	Ilan	Case Western Reserve	6-8-93	P	T	Cancer/ Brain	P	pAnti-IGF-1 (EBV derived)	NA	Case Western Reserve	12	0	P	P	P	P	P/FDA and NIH
9309-053	Cassileth, et. al.	U. of Miami	9-9-93	P	T	Cancer/ Small cell lung cancer	P	pBMG-Neo-hIL2/BPV derived (liposomes)	NA	U. of Miami	12	0	P	P	P	P	P/FDA and NIH
9309-054	O'Shaugn-essy	NIH	9-9-93	10-7-93	T	Cancer/ Breast	[9]	G1MD	PA317	GTI	18	0	P	P	P	P	P/FDA

Protocol #	PI(s)	Institution	RAC Approval Date	NIH Approval Date	M/T	Patients	Date First Patient Treated	Vector/ Gene	Packaging Cell	Vector Supplier	Maximum Patient Accrual	Patient Accrual To Date	In Vivo Evidence of Gene Transfer (Human)	Problems with Gene Transfer	Adverse Effects	# Deaths	Status (Open/ Closed)
9309-055	Kun, et. al.	St. Jude	9-9-93	10-7-93	T	Cancer/ Brain	P	G1TkSvNa (HS-tk cDna)	PA317	GTI	6	0	P	P	P	P	P/FDA
9309-056	Das Gupta, et. al.	U. of IL, Chicago	9-10-93	P	T	Cancer/ Melanoma	P	pZipNeoSv-IL2	GP+env AM12	U. of IL, Chicago	12	0	P	P	P	P	P/FDA and NIH
9309-057	Wong-Staal, et. al.	U. of San Diego, CA	9-10-93	P	T	HIV(+)	P	pMJT (hairpin ribozyme)	PA317	ND	6	0	P	P	P	P	P/FDA and NIH
9309-058	Economou, et. al.	UCLA	9-10-93	P	T	Cancer/ Melanoma	P	G1NaCvi2	PA317	GTI	30 (3 groups of 10)	0	P	P	P	P	P/FDA and NIH
Total # Patients												151					

Current data as of November 4, 1993.

	T	M	Total (T + M)
RAC Approved	36	23	59
NIH Director Approved	28	23	50
Categories of RAC-Approved Protocols			
Cancer	26	4	30
Cystic Fibrosis	4	1	5
SCID/ADA	1	0	1
Acute Hepatic Failure	0	1	1
Familial Hypercholesterolemia	1	0	1
Gaucher Disease	2	0	2
Bone Marrow Marking/Cancer	0	15	15
HIV(+)	2	2	4

TABLE LEGEND:

a	= Minor Modification
b	= Major Amendment
c	= Expedited Review
M	= Gene Marking Protocol
NA	= Not Applicable
ND	= Not Determined
P	= Pending
T	= Gene Therapy Protocol

ACRONYMS:

ADA	= Adenosine Deaminase
ABM	= Autologous Bone Marrow
ALL	= Acute Lymphocytic Leukemia
AML	= Acute Myelogenous Leukemia
ATCC	= American Type Culture Collection
BMM	= Bone Marrow Marking
BPV	= Bovine Papilloma Virus
CBSC	= Cord Blood Stem Cell
CF	= Cystic Fibrosis
CFU	= Colony Forming Units
CLL	= Chronic Lymphocytic Leukemia
CML	= Chronic Myelogenous Leukemia
EBV	= Epstein-Barr Virus
FDA	= Food and Drug Administration
FH	= Familial Hypercholesterolemia
G-CSF	= Granulocyte - Colony Stimulating Factor
GC	= Glucocerebrosidase
GTI	= Genetic Therapy, Inc.
GVH	= Graft versus Host
HHMI	= Howard Hughes Medical Institute
HIV	= Human Immunonodeficiency Virus
MA	= Microbiological Associates
MSKCC	= Memorial Sloan-Kettering Cancer Center
NBL	= Neuroblastoma
NHL	= Non-Hodgkin's Lymphoma
NIH	= National Institutes of Health
NSCLC	= Non-Small Cell Lung Cancer
PBL	= Peripheral Blood Lymphocytes
PBSC	= Peripheral Blood Stem Cell
SCID	= Severe Combined Immunodeficiency
SDRCC	= San Diego Regional Cancer Center
TGC	= Targeted Genetics Corporation
UCLA	= University of California, Los Angeles
USC	= University of Southern California

GLOSSARY

ADA	adenosine deaminase
AZT	azidothymidine
BCG	Bacille Calmette-Guerin
BPV	bovine papilloma virus
BRMP	Biological Response Modifiers Program
cDNA	complementary DNA
CF	cystic fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CFU	colony forming units
CSF	cerebral spinal fluid
CT	computerized tomography
CTL	cytotoxic T lymphocyte
DMRIE	1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl ammonium bromide
DOPE	dioleoylphosphatidylethanolamine
FACC	Fanconi anemia complementation C
FDA	Food and Drug Administration
GALV	gibbon ape leukemia virus
GC	glucocerebrosidase
GCV	ganciclovir
G-CSF	granulocyte colony stimulating factor
GM-CSF	granulocyte-macrophage colony stimulating factor
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSV-tk	herpes simplex virus thymidine kinase
IL	interleukin
IRB	Institutional Review Board
ml	milliliters
MoMuLV	Moloney murine leukemia virus
MRI	magnetic resonance imaging
NIH	National Institutes of Health
neo^R	neomycin resistance
NIH Guidelines	National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules
OPRR	Office for the Protection of Research Risks
ORDA	Office of Recombinant DNA Activities
pg	picograms
PGK	phosphoglycerate kinase
PI	principal investigator
Points to Consider	Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA into the Genome of Human Subjects
RAC	Recombinant DNA Advisory Committee
RCR	replication-competent retrovirus
UCSD	University of California, San Diego
VPC	vector producing cells

9312-059 Oldfield, Edward H. and Ram, Zvi; NIH, Bethesda, Maryland; *Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis.*

Date of RAC Approval: 12/2/1993
Date of NIH Approval: 1/20/1994

Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis

Scientific Abstract

Meningeal carcinomatosis occurs in 5% to 20% of all cancer patients. Most adult cases are due to breast or lung carcinomas. Over recent years the incidence of meningeal carcinomatosis has been reported to increase, perhaps since cancer patients survive longer with improved systemic therapy. Patients with meningeal carcinomatosis have an exceedingly poor prognosis. When maximal therapy is tolerated (intrathecal methotrexate and whole-brain irradiation) mean survival is limited to 6-7 months and fewer than 15% of the patients are alive at one year. In an attempt to improve this grim prognosis of patients with leptomeningeal carcinomatosis, we have developed a novel approach for the treatment of this disease. This approach makes use of recombinant DNA technology to transfer a sensitivity gene into the malignant cells seeding the leptomeninges. This is achieved by direct intrathecal injection of cells that actively produce a retroviral vector carrying the herpes simplex thymidine kinase gene (HStk), which sensitizes the cells to the antiviral drug Ganciclovir (GCV). The intrathecally injected producer cells and vector particles can circulate in the CSF and infect cells that are actively synthesizing DNA. In the subarachnoid space, such cells are predominantly tumor cells. The HS-tk gene is incorporated into the genome of tumor cells and results in expression of the protein encoded by the gene. The enzymatic interaction between HS-tk and GCV leads to the production of toxic triphospates of GCV that interfere with DNA synthesis and result in the death of the tumor cells. Since the thymidine kinase enzyme which is normally present in mammalian cells has very low affinity for GCV, systemic toxicity related to this mechanism is not expected.

The proposed clinical trial will evaluate the dynamics of retroviral vectors in the subarachnoid space, assess the safety of this approach and evaluate its potential antitumor efficacy. This is a dose-escalating study where increasing numbers of producer cells will be injected into the ventricular system and the spinal subarachnoid space. Indications of antitumor efficacy will include monitoring of clinical symptoms, craniospinal MRI evaluation, and CSF analysis for cytology and tumor markers. A total of 20 patients will be enrolled in the various phases of the study.

This is the first clinical attempt to treat meningeal carcinomatosis by *in vivo* , intrathecal, genetic manipulation of the tumor's genome.

Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis

Non-technical Abstract

Meningeal carcinomatosis is a complication of systemic cancer where malignant tumor cells seed the coverings of the brain and spinal cord (leptomeninges). The infiltrating tumor occurs in 5% to 20% of all cancer patients, and most adult cases are due to breast or lung cancer. Patients with leptomeningeal carcinomatosis present with a variety of neurological symptoms corresponding to involvement of cranial and spinal nerves, and infiltration into brain and spinal cord tissues. The prognosis is extremely poor. When maximal therapy is tolerated (intrathecal chemotherapy and whole-brain irradiation) mean survival is limited to 6-7 months and fewer than 15% of the patients are alive at one year. In an attempt to improve this grim prognosis of patients with leptomeningeal carcinomatosis, we have developed a novel approach for the treatment of this disease. This approach makes use of recombinant DNA technology to make the tumor cells sensitive to an antiviral drug called ganciclovir (GCV). Sensitivity to the drug is achieved by transferring a foreign gene into the malignant cells. This gene is the thymidine kinase gene from the herpes simplex virus. Cells that produce mouse viruses, which had been genetically engineered to contain the thymidine kinase gene, are injected into the subarachnoid space (intrathecal space; the space that is filled with cerebrospinal fluid and is in contact with the tumor cells in the leptomeninges). The viruses transfer the gene into the tumor cells and 7 days later the patient is treated with GCV, which leads to death of the tumor cells. Since the thymidine kinase enzyme which is normally present in mammalian cells has very low affinity for GCV, systemic toxicity related to this mechanism is not expected.

The proposed clinical trial will evaluate the distribution of retroviral vectors in the subarachnoid space, assess the safety of this approach, and evaluate its potential antitumor efficacy. As the study progresses, increasing numbers of producer cells will be injected into the ventricular system (cerebrospinal fluid-filled cavities in the brain) and the spinal subarachnoid space. Indications of antitumor efficacy will include monitoring of clinical symptoms, craniospinal MRI scans, and CSF analysis for the presence of tumor cells and tumor markers. A total of 20 patients will be enrolled in the various phases of the study.

This is the first clinical attempt to treat meningeal carcinomatosis by *in vivo* , intrathecal, genetic manipulation of the tumor's genome.

Protocol

Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis

- Clinical Trial -

Surgical Neurology Branch
National Institute of Neurological Disorders and Stroke
N. I. H.

PROTOCOL ROSTER

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PROTOCOL SYNOPSIS

Title: Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis

Objective:

Primary Objectives: 1) Assess the dynamics of retroviral vectors in the subarachnoid space after intraventricular and spinal injection of vector-producer cells.
2) Evaluate the safety of the approach
3) Evaluate the antitumor efficacy of this approach in the treatment of leptomeningeal carcinomatosis.

Population: Adult patients (>18 y) with documented leptomeningeal spread of their primary cancer who have failed standard therapy for their disease.

Sample Size: 20 patients in four phases (see Dosage/Treatment)

Dosage/Treatment: Phase A - 3 patients with intraventricular injection of vector-producer cells. CSF sampling for vector titer measurements and ganciclovir therapy.
Phase B - 3 patients with combined intraventricular and spinal injections of vector-producer cells, CSF sampling for vector titer measurements and ganciclovir therapy.
Phase C - 4 patients with repeat injections (intraventricular and spinal) of vector-producer cells (based on vector titer measurements in phases I and II) and ganciclovir therapy.
Phase D - 10 patients treated with the optimal regimen (based on titer measurements in previous phases).

(At each phase, treatment may be repeated for the same patient).

Duration: 2 years

Endpoints: *Safety Endpoints:* Development of grade 4 toxicity not responsive to therapy in 2 of 3 patients in phases A-C and 4 consecutive patients in phase D would be an endpoint for this specific (and subsequent) phases.

Efficacy Endpoints: Clinical, radiographic, and cytologic evidence for eradication of the leptomeningeal disease within 3 months of follow up.

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ABSTRACT

Meningeal carcinomatosis occurs in 5% to 20% of all cancer patients(9, 18, 22). Among the adult population, most cases of meningeal carcinomatosis are due to breast or lung carcinomas. Over recent years the incidence of meningeal carcinomatosis has been reported to increase, perhaps since cancer patients survive longer with improved systemic therapy(1,4,5,10,15,17,18,22,28). Patients with meningeal carcinomatosis have an exceedingly poor prognosis. When maximal therapy is tolerated (intrathecal methotrexate and whole-brain irradiation) mean survival is limited to 6-7 months(7,18,30) and fewer than 15% of the patients are alive at one year (26). Meningeal involvement is not necessarily a premorbid phenomenon and in two thirds of cases, it occurs when systemic disease is stable or in complete remission (7,30).

In an attempt to improve this grim prognosis of patients with meningeal carcinomatosis, we have developed a novel approach for the treatment of meningeal carcinomatosis. This approach makes use of recombinant DNA technology to transfer a sensitivity gene into the malignant cells seeding the leptomeninges. This is achieved by direct intrathecal injection of cells that actively produce a retroviral vector carrying the herpes simplex thymidine kinase gene (HStk), which sensitizes the cells to the antiviral drug Ganciclovir (GCV). The intrathecally injected producer cells and vector particles can circulate in the CSF and infect cells which are actively synthesizing DNA. In the subarachnoid space, such cells are predominantly tumor cells. The HS-tk gene is incorporated into the genome of tumor cells and results in expression of the protein encoded by the gene. The enzymatic interaction between HS-tk and GCV leads to the production of toxic triphospates of GCV that interfere with DNA synthesis and result in the death of the tumor cells. Since the thymidine kinase enzyme which is normally present in mammalian cells has very low affinity for GCV, systemic toxicity related to this mechanism is not expected. This type of in vivo gene transfer has several unique features. First, these retroviral-vectors will only integrate into and express their genes in cells which are actively synthesizing DNA. Therefore, non-proliferating normal brain and spinal cord tissue should not acquire the HS-tk gene and will remain insensitive to GCV. Second, all the transduced tumor cells (and retroviral vector producer cells) will be killed by GCV treatment reducing the risk of insertional mutagenesis. Third, in this setting, delivery of the producer cells and viral vectors will be facilitated by the normally circulating CSF which bathes the whole surface of the CNS.

The proposed clinical trial will evaluate the dynamics of retroviral vectors in the subarachnoid space, assess the safety of this approach and evaluate its potential antitumor efficacy. Phase A consists of intraventricular injection of 2×10^9 HStk producer cells followed by GCV treatment 7 days later for two weeks. In phase B 2×10^9 HStk producer cells will be injected intraventricularly and intrathecally in the lumbar subarachnoid space (total of 4×10^9 HStk cells). In phase C, intraventricular and intrathecal injection will be given twice prior to GCV administration (8×10^9 HStk cells). CSF samples will be analyzed for vector titers at multiple time points and, if no toxicity is encountered, 10 patients will be treated in phase D of the study with the same parameters as in phase C. Indications of antitumor efficacy will include monitoring of clinical symptoms, craniospinal MRI evaluation, and CSF analysis for cytology and tumor markers.

This is the first clinical attempt to treat meningeal carcinomatosis by *in vivo* genetic manipulation of the tumor's genome.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background/Current Treatment

Carcinomas of the breast and lung are the leading causes of cancer-related death in women in the United States. These cancers are also the leading cause of leptomeningeal carcinomatosis (LMC) (2,29). It has been shown that 35-40% of all breast cancer patients have CNS involvement at some point in their disease and that 5% of breast cancer patients have leptomeningeal involvement (20,21,24,27,30). Approximately 2,000 women will have leptomeningeal carcinomatosis as a result of their primary disease. Leptomeningeal involvement can, less frequently, be the initial presentation of the primary disease (12). Other forms of cancer such as lymphoma, leukemia, and ovarian cancer, have also been reported to metastasize to the leptomeninges. Primary CNS malignancies, such as medulloblastoma and ependymoma, are frequently associated with leptomeningeal seeding in the form of drop metastases to the lumbosacral subarachnoid space and cauda equina leading to treatment failure despite adequate control of the primary tumor.

Although visceral metastases from cancers are often associated with prolonged survival, meningeal involvement carries a very poor prognosis. Untreated patients, and patients unresponsive to treatment, survive less than two months (22,29,30). Patients who are able to tolerate optimal therapy (intrathecal methotrexate and whole-brain irradiation) survive for a mean of 6 to 7 months (7,18,29)while only 15% are still alive at one year (26). Meningeal involvement with cancer is not always a premorbid phenomenon and in two thirds to three quarters of cases with LMC, the systemic disease is stable or in complete remission (7,29). Effective therapy in these patients may extend survival and reduce morbidity.

The clinical presentation of LMC is one of progressive neurological deficits followed by the ultimate demise of the patient. Symptoms such as headaches, changes in mental status, and seizures indicate cerebral involvement and occur in up to 50 % of patients with LMC. Cranial nerve symptoms occur in 38% of patients. Symptoms of spinal root involvement include pain, paresthesias, and weakness occur in 70% of patients and are distinct from spinal bony or epidural metastasis. The treatment of these progressive neurologic deficits with multiple cranial nerve involvement, dementia, and often severe, deep pain, represents a major therapeutic challenge(8,18,26,29).

The interval from initial presentation of the primary cancer to the development of leptomeningeal carcinomatosis varies from 5-90 months. A disturbing aspect of this presentation is that, in a number of patients, it occurs when the peripheral tumor is in remission or has been relatively contained. Thus, there is a subset of patients in whom the primary active component of their disease is solely within the CNS. This subgroup of patients can potentially be cured if the leptomeningeal involvement could be eradicated.

Standard therapy for LMC includes radiation and chemotherapy. However, cancer is commonly radiation-resistant when it has spread to the leptomeningeal space, and significant side effects, such as severe bone marrow depression, may occur. There are a limited number of chemotherapeutic agents that can be used in the intrathecal compartment. Among the most well known and studied are methotrexate, cytosine arabinoside and thiotepa (3,7,11,13,23,25,26,29). Methotrexate is considered the most effective treatment for leptomeningeal spread of various tumors, including breast cancer (23). Long term use of methotrexate, particularly in patients who have received radiation, is associated with leukoencephalopathy, which can be detected by radiographic as well as cognitive testing. These changes are dose-dependent.

Even with an aggressive approach of radiation and intrathecal chemotherapy, extension of survival is marginal.

1.2 Strategy of Gene Therapy

Gene transfer in the subarachnoid space has several advantages of safety and potential efficacy. First, retroviral vectors only integrate, and therefore express, vector genes in proliferating cells. In the subarachnoid space, the tumor seeds comprise the most mitotically active cells. Among the normal tissues, only the choroid plexus, macrophage-derived cells and endothelial cells are at risk of being transduced. Therefore, the possibility of specific and preferential transduction of tumor cells is enhanced. Second, the CNS, including the subarachnoid space, is an immunologically privileged site due to the blood-CSF barrier. This should enhance survival of the xenogeneic murine cells and viral vectors and permit an increased transduction rate of the tumor. Subsequently, the producer cells and tumor cells that integrate and express HS-tk will be destroyed by GCV. In LMC, malignant cells, which grow as thin sheets on the leptomeninges, are exposed to the cerebrospinal fluid, (18) allowing circulating vector particles to reach the tumor cells and facilitating the distribution of the HStk gene into tumor cells.

1.3 Description of Viral Vector

G1TKSVNa is a retroviral vector derived from the Moloney murine leukemia virus (MoMLV). This vector contains 1) herpes thymidine kinase (HS-tk) gene cDNA that is transcribed from the viral LTR and 2) the bacterial neomycin resistance (NeoR) gene transcribed from an internal SV40 (simian virus 40) early promoter (LTR--HS-tk--SV--NeoR--LTR) in the G1 vector backbone (Genetic Therapy Inc., Gaithersburg, MD). This G1-based vector has been modified for increased safety by alteration of the gag start codon to a stop codon, and by elimination of viral sequences needed for formation of a replication-competent virus. This has been shown to decrease the potential for helper virus production from producer cells which contain the vector. No replication-competent virus has been detected in vectors administered to patients or following administration of the vector to animals or humans.

1.4 Preclinical Studies

(For detailed description of the pre-clinical efficacy and toxicity studies refer to appendix II)

Summary of Preclinical Efficacy Studies

In meningeal carcinomatosis retroviral vector-producer cells survive in the subarachnoid space long enough to distribute the vector in the CSF, to reach malignant tumor cells in the leptomeninges, and selectively infect and transfer a gene of interest to these cells. Gene transfer experiments with the lacZ gene and in vitro retroviral titer measurements showed that retroviral vectors survive in the CSF, retain their infectivity, and successfully transduce tumor cells. Significant antitumor effect was demonstrated by in situ, intrathecal, transduction with the herpes simplex-thymidine kinase gene followed with ganciclovir therapy in a rat model. Prolongation of survival was observed when ganciclovir was given systemically, but not intrathecally. Assessment of the antitumor efficacy of the G1Tk1SvN.7 HStk vector-producer cells demonstrated that intrathecal injection of the clone 7 cells resulted in significant prolongation of survival after i.p. GCV therapy. These results support the potential application of gene therapy using the HStk/GCV system for treatment of meningeal carcinomatosis.

Summary of Preclinical Toxicity Studies

Toxicity studies in rats indicated that no significant adverse effects were associated with intrathecal administration of the xenogeneic retroviral vector-producer cells, systemic GCV administration, or combination of the two. No transduction of normal leptomeningeal or subarachnoid structures throughout the brain and spinal cord occurred. Injection of HStk vector-

producer cells with or without GCV administration did not result in any observable toxicity, and histology showed no evidence of meningeal inflammation or parenchymal injury. Intraventricular injection of HStk and BAG producer cells resulted in transduction of the choroid plexus with some injury to the choroid plexus after GCV treatment.

Intraventricular administration of HStk (both clone 53 and clone 7) and ß-galactosidase retroviral vector-producer cells in rhesus monkeys did not produce adverse systemic or neurological effects, with or without GCV treatment. Necropsy data demonstrate transduction and injury of the choroid plexus in GCV-treated animals, however, this was not of clinical significance. Vector particles were detected along the whole length of the spinal canal (from the craniocervical region to the lumbar spine) 24 h after intraventricular cell injection, but not thereafter. These studies suggest that intraventricular and intrathecal injections of HStk vector-producer cells and subsequent GCV treatment are safe The limited period during which retroviral titers could have been detected in the CSF suggest that the vector-producer cell dose will need to be increased or multiple injections of producer cells given for optimal treatment.

1.5 Clinical Experience

Experience with Retroviral-mediated Gene Transfer Into Humans

A number of retroviral-mediated gene-marking (e.g. transduction of marrow cells in patients undergoing bone marrow transplantation) and therapy experiments (e.g. insertion of cytokine genes into autologous tumor in an effort to vaccinate patients) are currently being performed in humans. No untoward side effects related to retroviral-mediated gene transfer have been observed in any patient.

Eight patients with malignant brain tumors (6 recurrent glioblastoma, 1 multifocal glioblastoma with intracerebral metastases, and 1 metastatic malignant melanoma) were enrolled in a trial of gene therapy to evaluate toxicity and antitumor efficacy in human tumors. All patients had failed standard therapy, which included surgery and radiotherapy, and, in some patients, chemotherapy. CT or MRI-guided stereotaxis was used to distribute injections of murine HStk vector-producer cells throughout the contrast-enhancing portion of the brain tumors. Each patient received $5x10^8$ to $1.0x10^9$ producer cells in 5-10ml ($1.0x10^8$ cells/ml). Seven days after cell injection, ganciclovir (5 mg/kg, BID) was administered iv for 14 d. MR scans were performed frequently at the early stages of the treatment and at 2 wk intervals thereafter. PET scans, using FDG, were performed before and immediately after treatment in patients in whom PET identified a hypermetabolic tumor before treatment. Five of the 8 patients had evidence of an antitumor response. This was characterized by a decrease in tumor size (n=3) and/or acute changes in its consistency (micro and macrocysts, reduced gadolinium enhancement, n=5). After the initial response, a transient increase in size of the region of gd-enhancement occurred in 2 patients 2 wk after finishing treatment without any accompanying symptoms. Enhancement of the injection tracts was also evident. This spontaneously reversed 2-3 wk later (4-5 wk after completion of treatment) and is believed to represent a transient, self-limiting, inflammatory response. Three patients with rapidly enlarging glioblastoma progressed despite treatment. Biopsies obtained from 2 of these patients showed viable tumor cells, focal necrosis, an inflammatory response, and mild lymphocytic infiltrates. The injection of the murine cells and ganciclovir therapy were well tolerated by all patients and no adverse effects were observed.

1.6 Objectives

1) Assess the dynamics of retroviral vectors in the subarachnoid space after intraventricular and spinal injection of vector-producer cells.
2.) Evaluate the safety of this therapeutic approach.
3.) Evaluate the antitumor efficacy of this approach in the treatment of leptomeningeal carcinomatosis.

The above objectives will be pursued in the initial dose-escalation phases (A-C) of the proposed clinical trial as well as in the final phase of the trial. Determination of the objectives will be by serial clinical examination, serial CSF evaluation for cytology, vector titers, and tumor markers, routine laboratory evaluations, and repeat craniospinal MRI for evaluation of macroscopic tumor burden and meningeal enhancement.
See Appendix V for details.

2.0 Study Design

2.1 Overview

This is a Phase (I) single site, dose escalating, unblinded study of intrathecal transfer of the herpes virus thymidine kinase gene and ganciclovir therapy in patients diagnosed with leptomeningeal carcinomatosis from a metastasizing cancer.

2.2 Number of Patients

The number of patients for each phase of the study was determined to provide information (safety and vector dynamics in the subarachnoid space) before each subsequent phase is initiated. A total of 20 patients will be treated. (see section 3.2).

2.3 Patient Management

Patients will be treated as inpatients at the Clinical Center, Surgical Neurology Branch, NINDS, National Institutes of Health. Potential study patients who demonstrate an interest in participating in the study will be scheduled for a screening evaluation to determine their eligibility. Upon discharge, patients will be seen as outpatients (every 2 weeks during the first two months and monthly thereafter for one year and will be closely monitored for adverse effects or changes in clinical status. Follow-up for survival status will continue until death or until a final date for follow-up cessation is determined. Patients may receive repeat treatment(s) if they show disease progression after an initial response to therapy (see Section 9).

2.4 Study Duration

We expect to treat all 20 patients within one year. The duration of the study will be two years.

3.0 Study Treatment (Appendix V)

3.1 Method of Administration

3.11 *Phase A* HStk-retroviral vector-producer cells will be injected into the right lateral ventricle via an Ommaya reservoir (single injection). (3 patients)

3.12 *Phase B* HStk-retroviral vector-producer cells will be injected into the right lateral ventricle via an Ommaya reservoir (single injection) and into the lumbar subarachnoid space via a spinal catheter (single injection). (3 patients)

3.13 *Phase C* HStk-retroviral vector-producer cells will be injected into the right lateral ventricle via an Ommaya reservoir (repeat injections before GCV therapy) and into the lumbar subarachnoid space via a spinal catheter (repeat injections before GCV therapy). (4 patients)

3.14 *Phase D* 10 patients will be treated with the same regimen as in *Phase C* (or any previous phase if safety endpoints occur).

3.2 Dosing Regimen

The dosing regimen is summerized in Table 11 for phases A - D.

3.3 Risks, Hazards, and Discomforts

3.31 Potential Surgical Complications

Ommaya Reservoir Placement

A CSF reservoir consists of a small bubble-type reservoir and ventricular catheter. The placement of the CSF reservoir is a minor and routine neurosurgical procedure. It can be done using either general anesthesia or local anesthesia. The procedure requires shaving the hair over the right frontal portion of the head, just in front of the coronal suture. The area is then prepped and draped in a sterile fashion. The skin is infiltrated with local anesthesia and a small incision is made just in front of the coronal suture and 3 cm to the right of the midline. A burr hole opening in the skull is made and a catheter is then placed into the lateral ventricle on that side. The catheter is then attached to the reservoir and the skin incision closed. The risks of this procedure are quite small. There is a minor risk that the catheter may damage the brain through which it passes. In addition, there is a small risk of infection. Patients will receive perioperative antibiotics during placement of the CSF reservoir. The location of the CSF reservoir will be confirmed by CT scan.

After placement of the CSF reservoir, patients will be allowed to recover for 48 hours. Prior to administration of the murine producer cells, a CSF sample will be taken from the CSF reservoir and tested for the presence of malignant cells.

The surgical procedure (Ommaya reservoir placement) carries a small risk of intracerebral or intraventricular hemorrhage which can cause loss of neurologic function, as well as the usual risks of anesthesia and infection with attendant non-neurological complications and death. The degree of risk depends on the preoperative condition of the patient and associated diseases (e.g. ischemic heart disease, renal failure, COPD etc.) however, the risks are generally less for minor procedures such as Ommaya reservoir placement under local anesthesia.

Patients with malignant tumors have a significant predisposition to a variety of superimposed infections because of their state of immune suppression, as previously described. The probability of postoperative wound infection is also slightly increased because of the placement of the foreign material of the Ommaya reservoir . Antibiotic therapy (Rocephine) will be given as a prophylaxis during the surgical interventions and specific infections will be treated as needed.

Lumbar Catheter Placement

Percutaneous lumbar drainage of the subarachnoid space by an indwelling catheter placed at the bedside has been well described (16), and is a standard neurosurgical technique. The risks associated with CSF drainage by a small subarachnoid catheter are minimal. Some patients may develop a headache or backache following placement of the drain and prolonged headaches may develop in 1 to 5 % of patients. The headaches are usually relieved by bedrest and analgesics. If the headaches persist after removal of the catheter and last longer than one week it is possible to perform a "blood patch" (16). Catheters have been maintained for extended periods with minimal risk of infection. For a catheter maintained less than one week, the incidence of infection is less than 2 %.

3.32 Potential Treatment Complications

Chemical Meningitis

Despite the lack of a meningeal reaction in our animal models, meningitis or meningitis-like symptoms may develop secondary to injection of the vector-producer cells into the subarachnoid space. Such symptoms are expected to be self-limiting and ameliorated with symptomatic care (analgesics). However, such a reaction may be severe and could produce permanent neurological deficits or death. When indicated, additional CSF sampling may be required to rule out infection.

Hydrocephalus

Patients who develop hydrocephalus after initiation of the protocol with symptomatic increased intracranial pressure will be treated with CSF diversion via the Ommaya reservoir or, alternatively, through the lumbar drain if the increased intracranial pressure results from a communicating hydrocephalus, in which there is no obstruction to flow of the CSF in the ventricular system or basal subarachnoid space. Short term treatment of communicating hydrocephalus by lumbar CSF diversion is a standard neurosurgical treatment with minimal risk. If hydrocephalus persists, long term treatment with placement of a subcutaneous ventriculo-peritoneal shunt will be performed. The conversion of an Ommaya reservoir to a shunt system is a minor and routine neurosurgical procedure. To minimize the possibility of murine retroviral vector-producer cell transfer from the subarachnoid space to the peritoneal space, treatment with GCV will be completed prior to the internalization of the ventriculo-peritoneal shunt, if this is required.

3.33 Ganciclovir Administration

The GCV used in this trial will be purchased from Syntex corporation (Palo Alto, CA). GCV is an FDA approved drug for the treatment of cytomegalovirus (CMV) retinitis in immunocompromised individuals. The drug is administered by intravenous infusion over one hour at a dose of 5 mg/kg of body weight twice daily for 14 days.

Our experience with the use of GCV for the treatment of humans as a method to destroy herpes TK gene-transduced human cells in the "Intratumoral Transduction of Brain Tumor" protocol demonstrates no specific risks or complications of therapy in the 8 patients treated since initiation of the protocol. GCV crosses the blood-brain barrier. The cerebral spinal fluid (CSF)/plasma ratios have been estimated in three patients at various time points with ratios ranging from 0.24 to 0.7 (0.31-0.68 μg/ml in the CSF and 0.44-2.20 μg/ml in the plasma). Peak plasma levels have been documented to reach as high as 9 μg/ml. These CSF and plasma levels are expected to be within the range of GCV levels necessary to kill the HS-tk transduced tumor cells based on *in vitro* studies (0.5 μg/ml will prevent growth of HS-tk-transduced tumor cells). If the patient has evidence of renal impairment, the dose will be adjusted as suggested in the GCV monograph.

The most common side effects are granulocytopenia (absolute neutrophil count (ANC) of <1000 cells/mm^3) in 40% of patients and thrombocytopenia (<50,000 platelets/mm^3) in 20%. However, these data were collected in immunosuppressed, CMV-infected AIDS patients, who due to opportunistic infections and concomitant drug therapy, may have been more susceptible to marrow suppression than our patient population. The actual risk to our patient population is unknown. In the brain tumor protocol patients, one patient required administration of platelets before stereotaxic injection of producer cells because of thrombocytopenia after chemotherapy. He had no significant decrease in platelet count during GCV treatment. No granulocytopenia has been encountered with a total of 8 patients treated to date. Each patient will be closely monitored for the development of granulocytopenia or thrombocytopenia. The development of an ANC of <500 cells/mm^3 or a platelet count of <25,000 platelets/mm^3 will require dose interruption until the ANC >750 cells/mm^3 and platelet count >40,000 platelets/mm^3.

Other side effects, which occur in approximately 2% of patients include anemia, fever, rash

and abnormal liver function. Fever and chills will be treated with acetaminophen (650 mg every 4 hours) and rash with diphenhydramine (50 mg every 6 hours).

There are a number of other possible toxicities related to GCV administration. These toxicities have generally occurred in immunosuppressed, ill individuals receiving long term GCV administration. Our treatment protocol will use a dose (10mg/kg/day) that is known to be tolerated in non-infected patients and the GCV will be administered for only 14 days. These differences in patient population and treatment duration should minimize the likelihood of the development of the toxicities cited.

4.0 Patient Selection

4.1 Inclusion Criteria

Patients must meet the following criteria within 30 days prior to study entry (Day 0) unless otherwise noted:

4.11 Men or women, age $\geq$ 18 years of age
4.12 Established diagnosis of LMC confirmed by CSF cytology
4.13 Only patients who have failed standard therapy for LMC will be considered for the protocol. Failure will be defined as progression of neurological signs or symptoms, progression of leptomeningeal findings on MRI scan, or evidence of recurrence or progression by CSF cytology or tumor marker studies.
4.14 Adequate baseline organ function as assessed by the following laboratory values within 30 days prior to study entry (day -30 to 0):

Adequate renal function with estimated creatinine clearance > 50 ml/min. This will be determined by serum creatinine and 24-hour urine creatinine obtained concurrently.

Adequate liver function with SGOT, SGPT and alkaline phosphatase $\leq$ 5 times the ULN [if transaminases > than the upper limit of normal (ULN). Patients will have a hepatitis B surface antigen (HBsAG) test prior to study entry. Patients may not enter the study if HBsAG is positive.

PT and PTT not more than 1.5 times the ULN
Hemoglobin $\geq$ 8.0 g/dl (patients cannot be transfusion dependent)
Platelets $\geq$ 75,000/mm^3
WBC > 2,000/mm^3
Absolute Neutrophil Count (ANC) $\geq$ 1,000/mm^3
Serum Amylase $\leq$ 1.5 times the ULN
Bilirubin $\leq$ 2.5 mg/dL
Triglyceride < 400 mg/dl
4.15 Ability to give informed consent
4.16 Normal cardiac function by history and examination
4.17 Resting transcutaneous oxygen saturation > 90% on room air

4.2 Exclusion Criteria

Patients who meet any one of the following criteria will be excluded from study entry:

4.21 Pregnant or lactating females [all patients must practice adequate birth control and females of child-bearing potential must have a negative serum beta-HCG pregnancy test (within Day -7 to Day 0)].
4.22 Acute infection: any acute viral, bacterial, or fungal infection which requires specific therapy. Acute therapy must have been completed within 14 days prior to study treatment.

4.23 Hepatitis-B surface antigen positive patients.
4.24 HIV-infected patients.
4.25 Acute medical problems such as ischemic heart or lung disease that may be considered an unacceptable anesthetic or operative risk.
4.26 Patients with any underlying conditions which would contraindicate therapy with study treatment (or allergies to reagents used in this study).
4.27 Patients with prior ventriculo-peritoneal shunting procedure for hydrocephalus or who demonstrate signs, symptoms, or radiographic evidence of clinically significant hydrocephalus prior to treatment.

4.3 Concomitant Medication and Treatment
All medications or treatments will be kept to a minimum and recorded.

4.4 Medications and Treatments Not Allowed
Patients enrolled in the protocol will not be allowed to take any medications with established antitumor effects.

4.5 Potential Risks and Discomforts Related to Evaluations
Baseline and follow-up examinations will include blood tests, a lumbar puncture, aspirations of CSF from the Ommaya reservoir, and MRI scans of the brain and spinal cord.

Risks of Blood Drawing
Routine blood drawing may cause discomfort or temporary pain at the site of the needle entry may be the routine blood studies. There is remote risk of fainting and local infection.

Risk of Lumbar puncture
Lumbar puncture may be associated with discomfort or temporary pain at the site of the needle entry for the spinal tap or headaches immediately after the procedure. There is a remote risk of local infection, meningitis, or epidural or intradural hemorrhage.

Risk of Ommaya Reservoir Aspiration
Discomfort associated with tapping an Ommaya reservoir is minimal, probably less than for routine venipuncture. Aspiration of Ommaya reservoir for CSF may be associated with remote risk of infection of the hardware, ventriculitis, or obstruction of the ventricular catheter. Each of these may require removal or replacement of the Ommaya reservoir.

Risks of MRI
MRI is a painless procedure that is very safe for most patients. A small proportion of people develop short-lived reactions during the gadolinium administration including nausea, headache, hot flashes, and heart palpitations. These symptoms usually resolve spontaneously within minutes. Patients at risk for injury from MRI are those with pacemakers, cerebral aneurysm clips , or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible metallic foreign bodies in the eye. Patients at risk for injury by MRI scanning, will undergo CT scanning instead.

5.0 Clinical and Laboratory Evaluations

Appendix V is the Schedule of Evaluations Table

5.1 Screening and Baseline Evaluations

The following clinical and laboratory evaluations will occur within Study Days -30 to 0 unless otherwise noted. Screening evaluations, or evaluations that determine patient eligibility, are

noted. These screening evaluations must be reviewed prior to study treatment.

5.11 Objective Signs and Symptoms: include vital signs (blood pressure, pulse, temperature and respirations) weight and height. (Screening and repeat daily in inpatients).

5.12 History and Physical Examination: Screening, repeat targeted physical examination on Day 0

5.13 Chest X-Ray: CXR within the last 30 days to exclude acute inflammatory process, unless new symptoms suggest that this be repeated prior to dosing (Screening). Presence of lung metastases do not exclude patients from enrollment in the protocol.

5.14 Electrocardiogram (ECG): ECG must be normal within 30 days unless new symptoms suggest this be repeated prior to dosing (Screening)

5.15 Urinalysis: Routine urinalysis including a 24-hour creatinine clearance (Screening and repeat Day 0)

5.16 Serum Chemistries: include uric acid, calcium, phosphorus, magnesium, amylase, triglycerides, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, and glucose (Screening and repeat Day 0)

5.17 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen (Screening and repeat Day 0).

5.18 Pregnancy Test: Serum pregnancy test must be negative within 7 days prior to Day 0, or between study days -7 and 0.

5.191 Freezing Specimens: Additional serum, plasma, and blood cells will be separated and frozen at baseline (day -1 to day 1, prior to treatment). See appendix IX for appropriate processing, storage/shipping of study materials.)

5.2 Interval Evaluations

5.21 Objective signs and symptoms: Vital signs (blood pressure, pulse, temperature and respirations) will be done three times daily while hospitalized at the NIH Clinical Center.

5.22 Physical Examination: Target physical exam will be done daily.

5.23 Serum and Urine Chemistries: uric acid, calcium, phosphorus, magnesium, amylase, triglycerides, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, and glucose will be done weekly during GCV treatment. Creatinine clearance will be checked before and after GCV treatment.

5.24 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests should include PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen will be done every three days.

5.25 Freezing Specimens: Additional serum, plasma, and cells will be separated and frozen at the conclusion of GCV treatment.

5.3 Post-Treatment Evaluations

5.31 Complete Neurological and Physical Examination: Complete neurological and physical examinations will be done by study physicians at each follow-up evaluation

5.32 Blood Chemistries and Hematology: Complete blood count (CBC), differential, platelets, prothrombin time, partial thromboplastin time, uric acid, calcium, phosphorus, magnesium, amylase, triglycerides, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, albumin, total protein, electrolytes, glucose will be done at regular follow-up intervals.

5.33 CSF Sampling: CSF samples from lumbar puncture and Ommaya reservoir aspiration will be obtained at predetermined follow-up intervals for cell count, glucose, total protein, cytology, appropriate marker studies, and vector titer.

5.34 MRI Scans: Gadolinium-enhanced MRI scans of the brain and spinal cord will be done at each follow-up evaluation.

5.4 Autopsy Request

All participants in the study will be informed that an autopsy will be requested to determine the extent of gene transfer into important organs such as marrow, liver, and spleen.

5.5 Considerations/Evaluations Upon Premature Withdrawal

Patients who are discontinued or withdraw before completing the study, but have completed at least 1 cycle of therapy will be asked to return to the clinic to be evaluated according to Section 5.3.

5.6 Retreatment Criteria:

If initial response to the treatment was demonstrated, but there is residual disease or the leptomeningeal carcinomatosis recurs, repeat retreatment will be considered.

6.0 Treatment Modification and Discontinuation According to Level of Toxicity

The Table for Grading Severity of Adverse Experience (Appendix VI) will be used to achieve consistency in response to drug/treatment toxicities. Toxicities will be graded on a 1 - 4 grading scale. If a toxicity is experienced, the treatment level or dose will be modified as outlined below according the grade of toxicity observed.

6.1 Treatment Modification and General Management of Toxicities

For Grades 1, 2, and 3 toxicity symptomatic treatment with careful monitoring of the patients clinical condition will be performed. Such symptomatic treatment may consist of CSF diversion for hydrocephalus as discussed previously in section 3.32. Medical management of chemical meningitis using medications routinely used in neurosurgical practice for symptomatic relief is discussed below (6.11).

Development of Grade 4 toxicity which responds to therapy will be treated symptomatically as above. Any Grade 4 toxicity which is unresponsive to therapy will necessitate modification in the dose of vector-producer cells to the number of cells administered at a previous phase for the next patient.

6.11 Management of Specific Toxicities

Management of potential chemical meningitis from injection of the vector-producer cells into the subarachnoid space has been discussed in section 3.32 and consists primarily of symptomatic relief with analgesics or steroid therapy combined with surveillance for potential infection. Management of other specific toxicities were previously discussed. (See 3.3)

6.2 Symptomatic Therapy for Toxicity

Symptomatic therapy such as analgesics or other helpful therapy may be administered if deemed necessary by the participating investigators. All medications administered will be recorded in the appropriate Case Report Form.

7.0 Criteria for Discontinuation of Study Patients

7.1 Criteria for Treatment Discontinuation

Participation in this study can be discontinued for any of the following reasons:

o At the Patient's request: Any patient who wishes to withdraw from the study may do so but will be counseled that long-term follow-up for the effects of gene therapy are strongly desired by the medical facility and federal regulatory groups.

o A major, unexpected, or life-threatening toxicity requiring that treatment be discontinued

o Generalized impairment or mental incompetence which would render the patient unable to understand his/her participation in the study. In such cases continuation will be determined by the person designated by durable power of attorney.

o If, in the Investigator's medical judgment, further participation would be injurious to the subject's health or well-being.

o Administrative reasons, such as subject non-compliance or a major protocol violation.

o Pregnancy

An explanation will be recorded for any patient that is taken off treatment and the appropriate Case Report Form completed.

7.2 Criteria for Study Discontinuation

Patients should understand that once they have completed (at least one cycle of therapy), they will be asked to be followed long-term. They can stop study treatment at any time (see section 7.1), however they will still be considered on study until the study has been terminated.

8.0 Adverse Experiences

Appendix VII is Adverse Experience Reporting

Depending on the extent of the adverse experience, adverse events will be reported to the NIH Institutional Review Board (IRB), Institutional BioSafety Committee (IBC) and to Genetic Therapy, Inc. (sponsoring company) as specified in Appendix VII.

Adverse reactions or deaths requiring immediate reporting will be made by telephone to

(301) 590 2626 (available 24 hours a day, recorder after working hours).

The sponsor, Genetic Therapy, Inc. takes the responsibility of informing and preparing reports for the Recombinant Advisory Committee (RAC) through the NIH Office of Recombinant DNA Activities (ORDA) and to the FDA.

9.0 Evaluation of Response/Endpoints

9.1 Study Endpoints:

9.11 *Safety Endpoints:* Development of grade 4 toxicity not responsive to therapy in 3 patients in phases A-C and 4 consecutive patients in phase D are the endpoints for each specific (and subsequent) phases.

9.12 *Efficacy Endpoints:* Clinical, radiographic, and cytologic evidence for eradication of the leptomeningeal disease within 3 months of follow up. (See Appendix VIII for details)

9.2 Response Criteria:

Patients will be evaluated repeatedly throughout the study. Their neurological exam will be recorded twice daily during the initial 48 hours after both the injection of producer cells and infusion of GCV. Additionally, their neurological exam will be documented when they are discharged. Responses will be based on the following grading system:

Level I	Complete Response: No evidence of disease by cytology/tumor markers, MRI, and clinical exam.
Level II	Partial Response: A decrease in malignant cells remaining in the CSF by 50% and/or an improvement in neurological exam, and/or change in the MRI evidence of leptomeningeal involvement or tumor markers.
Level III	Stable disease and parameters of LMC (cytology/tumor markers, MRI, clinical exam).
Level IV	Progression of Disease

9.3 Required Documentation:

Gadolinium-enhanced MRI scans of the brain and spinal cord will be performed at 2, 6, and 12 weeks after therapy. Ventricular and spinal CSF samples will be obtained at 2, 6, and 12 weeks after treatment. These samples will be assessed for cytology and tumor markers.

10.0 Documentation, Record Keeping, CRFs

All documents relevant to the conduct of the study will be kept on file by the investigator for (five) years after all investigational use of product is discontinued and the FDA is so notified or until five years after a Product Licensing Application (PLA/ELA) is approved.

A separate release of medical information form will be signed by the study patient to facilitate access to the patient's medical records should the patient be hospitalized at another institution. A record will be kept of all patients considered (and screened) for the study and subsequently deemed ineligible. The reason for ineligibility will be recorded.

11.0 Data Collection and Study Monitoring

11.1 Data Collection

Case Report Forms (CRFs) will be utilized for each patient entered into the study. Study participants will NOT be identified by name on any study documents. Patients will be identified by a patient identification number (PIN) and a patient code list will be kept.

11.2 Study Monitoring

The GTI-designated person responsible for study monitoring of patient data will be permitted to contact and visit the investigator at regular intervals and will be allowed, on request, to review original documents such as consent forms, drug accountability records, case report forms, and pertinent hospital or clinic records for confirmation of study data provided that patient confidentiality is maintained in accord with local (IRB) requirements. It will be the monitor's responsibility to verify the adherence to the protocol and the completeness and accuracy of the data being entered on the CRFs.

12.0 Biostatistical Considerations

Not applicable

13.0 Ethical Considerations

13.1 Informed Consent

All study participants must sign an informed consent (see Appendix X). The investigators will inform all subjects as to the nature, aims, duration, potential hazards, and procedures to be performed during the study and that his or her medical records may be reviewed by the sponsors or the FDA. Patients will also be notified that they are completely free to refuse to enter the study or to withdraw from it at any time. The protocol will be discussed in detail with all potentially eligible patients. All revisions of the protocol will be reflected in the consent form and reviewed by the IRB.

13.2 Patient Confidentiality

All reports and patient samples will be identified only by a coded number to maintain patient confidentiality. All records will be kept confidential to the extent permitted by law. The investigators will keep a separate log of patients' codes, names, and addresses. Documents which identify the patient by name (informed consent) will be kept in strict confidence.

14.0 Criteria and Procedures for Protocol Modification and Study Termination

Modifications which may effect the safety of the study patient, or which may alter the scope of the investigation, the scientific quality of the study, the study design, dosages, duration of therapy, patient assessments (added evaluation that poses potential risk or inconvenience to the patient), number of patients, and patient eligibility criteria, may be made only after appropriate

consultation between the investigator, the IRB, and the sponsor.

If the consensus is to revise the current protocol, a formal List of Changes will accompany the amended protocol and these will be submitted to the FDA, the IRB, and other committees as required.

Both the sponsor and the investigator reserve the right to terminate the study at any time. If this becomes necessary, appropriate procedures for continuing the long-term follow-up requested by the regulatory agencies will be arranged after review and approval by both parties.

15.0 Publication of Data/Protection of Company Secrets

The investigator will agree to inform the sponsor of all study results. Any manuscript, abstract, or presentation will be made available to the sponsor and all participating study investigators for review prior to submission of manuscripts, abstracts, or scientific meetings. This will allow the sponsor to protect proprietary information and to provide comment based on information that may not yet be available to the investigator. Authorship will be determined by mutual agreement.

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INSTITUTE: NINDS

Informed Consent

STUDY NUMBER: _____ PRINCIPAL INVESTIGATOR: Edward H. Oldfield, M.D.

STUDY TITLE: Intrathecal Gene Therapy for the Treatment of Leptomeningeal Carcinomatosis
Phase A

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health. It is important that you read and understand several general principles that apply to all who take part in our studies: (a) taking part in the study is entirely voluntary; (b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others; (c) you may withdraw from the study at any time without penalty or loss of any benefits to which you are otherwise entitled. The nature of the study, the risks, inconveniences, discomforts, and other pertinent information about the study are discussed below. You are urged to discuss any questions you have about this study with the staff members who explain it to you.

Leptomeningeal carcinomatosis (LMC) is the spread of malignant tumor cells along the membranes that cover the brain and spinal cord. There is no effective treatment for this condition when it recurs after radiation therapy and chemotherapy. Therefore, we have developed a new experimental approach that takes advantage of knowledge gained in the fields of genetics and gene therapy. A virus that causes mouth sores and other types of infections is called herpes simplex. It can be treated with a drug called Cytovene. Herpes simplex is killed by Cytovene because the virus contains a specific gene called the Herpes-thymidine kinase gene (TK gene). The TK gene has been isolated in the laboratory and in experiments has been injected into tumor cells in the lining of spinal cords in rats. This causes the spinal cord tumor cells containing the TK gene to be killed when the rats are treated with Cytovene. Rats treated this way had slower progression of paralysis and survived longer than untreated rats with the same tumors of the spinal cord lining.

The purpose of this study is to gain information about whether or not this new approach is safe and effective in treating human tumors of the spinal cord lining. Although the findings in animals are encouraging, you will be one of the first humans to receive this investigational treatment for LMC. While we hope to gain information on the usefulness of this approach in humans, we cannot know whether your tumor will get smaller as a result of this experimental treatment. It is possible that this experimental treatment could make you worse.

WHAT IS GENE THERAPY FOR LMC?

We will attempt to change the genetic material of tumor cells in the lining of your brain and spinal cord by inserting the TK gene into the tumor cells. The way we will transport the TK gene into the tumor cells is to use a "vehicle" to carry the TK gene into the cells. An example is that if you want to move a passenger you need to put him in a car. The vehicle (or car) in this case is a different virus - a retrovirus that is found in mice - that has been inactivated so that it can not cause disease. We call this vehicle a "vector". We insert the TK gene (the passenger) into the vector and the vector carries the gene into your brain tumor cells. The vector/gene combination

is produced by "vector-producer" cells (little factories that produce the cars containing the passengers). When the vector/gene combination gets into your tumor cells it inserts itself into the cells' genetic material (chromosomes) and tells the tumor cells to begin making thymidine kinase (and thus behave like a herpes virus). We will also attach to the vector vehicle a bacterial gene which we think is harmless; this gene serves to mark the location of the TK gene in your body. When we give you Cytovene, the tumor cells containing the TK gene make harmful substances within themselves that cause the tumor cells to die. We do not expect normal spinal cord or brain cells to be injured because the vector/gene combination is attracted only to rapidly growing cells. Normal cells in the brain and spinal cord are not rapidly growing, but tumor cells are and therefore become targets for the vector/gene combination.

PRETREATMENT EVALUATION

You will be admitted to the hospital for approximately 1-2 weeks for the pretreatment evaluation and the various procedures. You will have a complete neurological and physical examination, blood, and urine tests to determine your overall physical health. The amount of blood drawn from you will be no more than three ounces in one day and will not exceed one pint over your stay at the hospital. You may experience some discomfort or temporary pain at the site of the needle entry for the routine blood studies. There is remote risk of fainting and local infection. In addition a spinal tap will be done for initial spinal fluid tests. You may experience some discomfort or temporary pain at the site of the needle entry for the spinal tap or headaches immediately after the procedure. There is a remote risk of local infection or meningitis (inflammation of the coverings of the brain or spinal cord).

You will also have x-rays of your chest and head and any other areas of the body as indicated by your medical examination and history. An electrocardiogram will be performed. Additional tests may be required depending upon your state of health and prior therapies. The blood tests will also include a test for the HIV (AIDS) virus. Women of childbearing age will also have a pregnancy test. Patients who are HIV positive and pregnant women will not be eligible for this protocol. We do not know the impact of experimental drugs on fetal development. Damage to cell growth may occur while you are being administered the drugs or after administration of drugs. Therefore, only those individuals who are using effective means of contraception will be permitted in this study.

An MRI scan of your brain and spinal cord will be done. MRI is designed to examine the changes in the nervous system that occur with tumor seeding of the lining of the brain and spinal cord. MRI is a new diagnostic tool that uses a strong magnetic field and radio waves to show structural and chemical changes in the brain and spinal cord tissues. This technique has been found more sensitive than x-rays to detect a variety of processes within the brain and spinal cord. In addition, MRI has the advantage of not using radiation. During the MRI study, a needle will be placed into a vein in your arm and an image-enhancing dye, gadolinium, will be administered

through that vein before obtaining a second set of scans. Each scan takes about 60 to 90 minutes. MRI scans will be repeated periodically after injection of the vector-producer cells to monitor the effects of this therapy on tumor growth.

TREATMENT PHASE

You will undergo 4 procedures in the following order: (1) surgery for placement of a reservoir device (Ommaya reservoir) with a catheter inserted into the spinal fluid cavity (ventricle) in the brain, (2) injection of vector-producer cells into the reservoir device, (3) placement of a spinal fluid catheter in the lower back for sampling spinal fluid for 7 days after cell injection, and (4) intravenous administration of the drug Cytovene. The treatment phase is expected to require a 3-4 week hospitalization.

If you show improvement after this treatment phase, but later show evidence of disease progression, we may consider repeating the cell injections and Cytovene administration during another hospitalization

Placement of Ommaya Reservoir:

Surgery will be performed under general anesthesia. A small area of the right frontal scalp will need to be shaved. A curved skin incision about 2 inches in length will be made in the scalp and a drill hole will be made in the skull. Through this hole a thin plastic tube will be passed into the fluid filled cavity, or ventricle, in the brain. This tube will then be connected to a plastic reservoir (Ommaya reservoir), roughly the size of a quarter, and the skin closed over this device so that it will lie entirely under the skin when the incision heals. Medications and vector producing cells can be injected into the reservoir with a hypodermic needle through the skin, thus providing access into the spinal fluid system. Spinal fluid samples can also be withdrawn through the reservoir using a hypodermic needle.

Injection of Vector-Producer Cells:

The vector-producer cells will be injected through the Ommaya reservoir. This will be done at the bedside in the hospital room. The skin over the reservoir will be cleaned sterilely and the cells, suspended in a small amount of liquid, injected through the skin and into the reservoir with a hypodermic needle and syringe. This will probably cause no more discomfort than drawing a blood sample through a vein in the arm.

Placement of Lumbar Spinal Fluid Drain:

In order to obtain frequent spinal fluid samples immediately after cell injections, a lumbar spinal fluid drain will be placed. This procedure is similar to a routine spinal tap except that a very thin, soft plastic tube is inserted through the spinal tap needle and is left in place connected to a drainage bag. This procedure is done at the bedside in the hospital room using local anesthesia. This drain will remain in place for 5-7 days and during this time you will need to

remain flat in bed. Although somewhat inconvenient, this spinal fluid drain will prevent the need to do several spinal taps a day to obtain the spinal fluid samples that we need to evaluate your treatment.

Cytovene administration:

Seven days after injection of vector-producer cells, Cytovene infusions will be started. During the administration of Cytovene into one of your veins, you will stay in a regular hospital room. You will undergo a repeat MRI scan before Cytovene administration. Cytovene will be administered into a vein in a slow drip lasting one hour. The drug will be given two times a day for 14 days.

FOLLOW-UP CARE AND EVALUATION

After you will finish the course of Cytovene you will be discharged from the hospital. Two weeks after discharge, you will be required to return to the NIH as an outpatient for follow-up studies. You will undergo neurological and physical examinations and laboratory tests, as were performed before your surgery, including a spinal tap and obtaining a spinal fluid sample from the Ommaya reservoir. An MRI scan of the brain will also be obtained. Following the initial visit, you will be seen as an outpatient at 2 weeks intervals for 2 months and monthly for 10 months. The number of visits after the first year will depend on the status of your tumor. We expect that we will receive one sample of blood per year for the remainder of your life to monitor the safety of this gene transfer methodology.

RISKS

Risks of MRI:

MRI is a painless procedure that is very safe for most patients. During the MRI study you will lie flat in a long metal cylinder, which people who fear closed spaces sometimes find a little frightening. Patients can be observed at all times by the operators and can be assisted if necessary. You will be moved out of the machine if requested. The thumping sound you will hear is the radio waves forming the images.

A small proportion of people develop short-lived reactions during the gadolinium administration including nausea, headache, hot flashes, and heart palpitations. These symptoms usually resolve spontaneously within minutes. A smaller group of patients are actually allergic to the dye and may develop a rash, hives, respiratory difficulty, and in extreme cases pulmonary and cardiac arrest and death. You will be closely monitored and if an allergic reaction develops, you will be promptly treated with epinephrine and breathing assistance if necessary. Both therapies are immediately available in the room where the MRI scan will be performed.

Patients at risk for injury from MRI are those with pacemakers, aneurysm clips (metal clips

on the wall of a large artery), or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible metallic foreign bodies in the eye. If you are at risk for injury by MRI scanning, you will undergo CT scanning instead.

Risks of Surgery for Placement of Ommaya Reservoir:

The surgical risks depend on the preoperative condition of the patient. Known risks associated with surgical placement of Ommaya reservoir include hemorrhage, local infection of the wound, meningitis, malfunction of the reservoir device which could require additional surgery, and death. The relative risks will be discussed with you in accordance with your condition and specific findings.

Risks of Inserting a Spinal Fluid Drain:

The risks for placement of a lumbar spinal fluid drain are similar as those for a routine spinal tap. There is risk of bleeding, infection, and injury to nerves to the legs. The risk of infection is higher than for a routine spinal tap, but remains small if the drain is removed after 7 days as we plan to do. There is some risk of discomfort or temporary pain at the site of needle insertion in the lower back, as well as headaches which can accompany any spinal tap.

Risks of the Vector/TK Gene Transfer:

Even though the vector-producer cells cannot grow and are considered harmless in humans, it is possible that events could occur within the cells that would permit the vector to grow and/or make the cells cancerous. Gene transfer using similar vectors has been used in adults and children. Since these experiments started in 1989, none of the people who have received cells into which genes had been transferred by vectors has developed any problems related to the gene transfer method. We believe these vectors are safe and are not a threat to you, other people, or to society.

This is the first experiment in humans that will involve the implantation of vector-producer cells into the spinal fluid. This method of treatment has 3 major potential problems. First, the vector may be passed into surrounding normal tissue in addition to tumor tissue. We have not found any evidence of problems in mice, rats, or monkeys due to the spread of vector to surrounding normal brain and spinal cord tissue or to other sites in the body. We believe that some of the surrounding blood vessel cells probably do have the vector, but the number is too small to result in significant adverse side effects. It is possible that bleeding and neurologic symptoms (headache, convulsions, stroke, paralysis) may develop with Cytovene.

Second, the vector-producer cells might persist in your body and cause cancer or other diseases. We expect your immune system to reject (kill) the vector-producer cells in 1-2 weeks. Thus, they should not be able to survive and grow in your body. In addition, we expect the Cytovene therapy will kill all cells with the vector, including the vector-producer cells. Therefore,

the vector-producer cells should not survive and the insertion of the vector should not result in new cancerous cells. Indeed, the potential risk of causing a new cancer seems very small because no cancers have been found in any of the experiments in which genes have been transferred into monkeys (since 1985) and humans (since 1989) using our vectors and conditions.

Third, the result of injecting vector-producer cells into the spinal fluid system may cause inflammation of the membranes covering the brain and spinal cord (meningitis) or impair normal circulation of the spinal fluid throughout the brain and spinal cord (hydrocephalus). Neither of these potential complications occurred in our animal experiments. Meningitis can be treated with medications. Treating hydrocephalus might require additional surgery to convert your Ommaya reservoir into a shunt, or device which would divert the spinal fluid into the abdominal cavity.

Additional Risks:

Finally, because this procedure is relatively new, it is possible that despite our extensive efforts, other unforeseen problems may occur including the possibility of death.

Risks of Cytovene Therapy:

Cytovene has been used extensively in humans to treat a number of infections including viral infections of the eye. We will be following the recommended dose for therapy that has been used to treat viral infections of the eye. The most commonly observed complication in people receiving Cytovene has been the development of decreased white blood cell and platelet counts. This could result in an increased risk of infection and bleeding. We will monitor these counts every other day (during Cytovene treatment) and the drug will be discontinued if the white blood count or platelet count drop significantly. Discontinuation of the drug results in normalization of the white blood cell and platelet numbers. Cytovene may cause permanent or temporary infertility and may be associated with birth defects. Therefore, women of childbearing age should use effective contraception during Cytovene therapy and men should use contraception during and for at least 90 days following Cytovene therapy. Pregnant women are not eligible for this protocol. Cytovene can cause cancer in animals. There is no information available to estimate the risk of this in humans.

DURABLE POWER OF ATTORNEY

You have an illness that at some point may impair your ability to think clearly and to make decisions. A Durable Power of Attorney is a process by which you appoint someone to make decisions about your medical care at the Clinical Center should you become, and only if you become, unable to make these decisions yourself. Such people might include a husband, wife, other family member or close friend. It is important that this person know your moral, religious

and/or personal preferences in research and medical care. The Clinical Center Durable Power of Attorney form has no legal standing outside the NIH but helps us give you good medical care and assures that your preferences in research and medical care are respected, if you should be unable to give informed consent.

A TENTATIVE AGREEMENT TO AN AUTOPSY

In case of death during or after your participation in this research study, we ask you for a tentative agreement to an autopsy. Enrollment in this protocol implies that you agree in principle to have a post-mortem examination. This will provide us with an opportunity to learn more about the effect of our treatment of your tumor.

ALTERNATIVE TREATMENTS

You have been offered the opportunity to participate in this study after your tumor recurred despite appropriate standard therapies for your disease. There are no other standard treatments that were shown to have significant effects in patients with your disease. A variety of experimental studies for the treatment of tumor seeding of the spinal cord lining are conducted in medical centers around the world, but the efficacy of their approaches is as yet unknown. In addition, there is always the possibility for you to decline any further treatment for your disease.

CONFIDENTIALITY OF DATA

This clinical procedure has attracted a great deal of attention from the media. We will make every effort to protect the confidentiality of you and your family. However, because of this media interest there is a greater risk than usual that information concerning your treatment will appear publicly without your consent.

You are free to withdraw your consent to participate in this study at the NIH and seek care from any physician at any time. This will involve no loss of benefits and will not penalize your care in any way.

As part of your participation in this study, it will be necessary to test your blood for the presence of antibodies to the Human Immunodeficiency Virus (HIV), the virus that causes Acquired Immune Deficiency Syndrome (AIDS). In order to perform the test, a small amount of blood (approximately 2 teaspoons) will be withdrawn from one of your arms with a needle. You may experience some slight discomfort at the needle entry site and there may be some bruising. In addition, there is a very small risk of your fainting or of infection at the needle entry site. If your test results are found to be positive, or if you are otherwise diagnosed as having AIDS, you should be aware of the following Clinical Center HIV Testing Policy:

1. Your physician will notify you promptly of the HIV test results.

2. Your physician and/or the Clinical Center HIV counselor will offer you, and any current and/or ongoing sexual partner(s) (spouses are generally considered to be current or ongoing sexual partners) or needle-sharing partner(s) you identify, information on the meaning of the test results and how to prevent the spread of the infection.

3. Because the virus may be transmitted in several ways, it is important that you inform sexual and/or needle-sharing partner(s) that any, or all, of them may have been exposed to the HIV virus and encourage them to be tested. If you request it, staff at the Clinical Center will assist you in notifying your partner(s) and arrange counseling for them through an HIV counselor.

4. The results of your HIV test and/or documentation of the diagnosis of AIDS will become a part of your Clinical Center medical record and, as such, will be protected from unauthorized disclosure by the Federal Privacy Act of 1974. In general, access to your medical record will be restricted to those health care professionals directly involved in your care or in the conduct of ongoing biomedical research, and information is not usually released to other third parties without your permission or that of your designated representative. However, there are some particular routine uses of such information of which you should be aware.

   a. If you are unwilling or unable to notify your partner(s), the Clinical Center is responsible for attempting to contact and inform them of their possible exposure to the virus. Reasonable attempts will be made to protect your identity including withholding your name when notifying any partner(s) of their possible exposure. Some notification or counseling of current and/or ongoing partners may be carried out through arrangements with, or referral to, local public health agencies.

   b. A summary of your care at the Clinical Center will be sent to the physician who referred you here for treatment.

   c. The Clinical Center may report certain communicable diseases, including AIDS and symptomatic HIV infection, to appropriate State and Federal government agencies.

If you have any questions regarding the HIV testing or the information provided above, you are encouraged to discuss them with your physician and/or a Clinical Center HIV counselor (496-2381).

STUDY NUMBER

OTHER PERTINENT INFORMATION

1. **Confidentiality**. When results of a study such as this are reported in medical journals or at meetings, the identification of those taking part is withheld. Medical records of Clinical Center patients are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

2. **Policy Regarding Research-Related Injuries**. The Clinical Center will provide short-term medical care for any physical injury resulting from your participation in research here. Neither the Clinical Center nor the Federal government will provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.

3. **Payments**. If you are a patient, you are not paid for taking part in NIH studies. Exceptions for volunteers will be guided by Clinical Center policies.

4. **Problems or Questions**. Should any problem or question arise with regard to this study, with regard to your rights as a participant in clinical research, or with regard to any research-related injury, you should contact the principal investigator Edward H. Oldfield, M.D. or these other staff members also involved in this study; Zvi Ram, M.D., Building 10, Room 5D37 Telephone: 496-2921. Bethesda, Maryland 20892 Between 5 pm and 8 am, weekends and holidays, contact the Neurology on-call physician on 496-4567

5. **Consent Document**. It is suggested that you retain a copy of this document for your later reference and personal records.

COMPLETE APPROPRIATE ITEM BELOW, A OR B

A. **Adult Patient's Consent**

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient & Date Signed

B. **Parent's Permission for Minor Patient.**

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s) & Date Signed

(if other than parent, specify relationship)

Signature of Investigator & Date Signed

Signature of Witness & Date Signed

PATIENT IDENTIFICATION

[622]

CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY
• Adult Patient or • Parent, for Minor Pa

NIH-2514-1 (10-84)

CURRICULUM VITAE

EDWARD HUDSON OLDFIELD, M.D.

PERSONAL DATA

Born: November 22, 1947
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EDUCATION

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EXPERIENCE

1973 - 1974	Internship: Surgical, Vanderbilt University Hospital, Nashville, TN
1974 - 1975	Residency: General Surgical, Vanderbilt University Hospital, Nashville, TN
1975 - 1976	Neurology: Junior Registrar, National Hospital for Nervous Disease, Queen Square, London, England
1976 - 1980	Neurosurgical Residency, Vanderbilt University Hospital, Nashville, TN
1980 - 1981	Private Neurosurgical Practice, Lexington Neurosurgical Associates, Lexington, KY
1981 - 1983	Senior Staff Fellow, Surgical Neurology Branch - National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD
1983 - 9/1984	Deputy Chief for Clinical Neurosurgery, Surgical Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD
9/1984 - Present	Chief, Clinical Neurosurgery Section, Surgical Neurology Branch, NINDS, National Institutes of Health, Bethesda, MD
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9312-060 Sobol, Robert E. and Royston, Ivor; San Diego Regional Cancer Center, San Diego, California; *Injection of Colon Carcinoma Patients with Autologous Irradiated Tumor Cells and Fibroblasts Genetically Modified to Secrete Interleukin-2.*

Date of RAC Approval: 12/2/1993

SCIENTIFIC ABSTRACT

The overall objective of this study is to perform a Phase I Clinical Trial in colon carcinoma patients of cytokine gene transfer comprising subcutaneous immunizations with a mixture of irradiated autologous fibroblasts genetically modified to express the gene for IL-2 and irradiated autologous tumor cells. Cytokine gene transfer has resulted in significant anti-tumor immune responses in several animal tumor models. In these studies, the transfer of cytokine genes into tumor cells has reduced or abrogated the tumorigenicity of the cells after implantation into syngeneic hosts. We have successfully induced anti-tumor immunity in a model of colorectal carcinoma by immunization with a mixture of irradiated tumor cells and IL-2 transduced fibroblasts. Immunization with a mixture of irradiated tumor cells and IL-2 transduced cells induced systemic anti-tumor immunity capable of rejecting a subsequent live tumor cell challenge. Repeated immunizations with a mixture of irradiated tumor cells and IL-2 transduced fibroblasts abolished established, visible tumors in a subset of the treated animals. Colorectal carcinoma is one of the most common cancers in the United States and Europe with an annual incidence of greater than 150,000 in the U.S. Most patients are treated with tumor resection and do not have clinically detectable tumor following surgery. However, the majority of patients have microscopic metastases and eventually relapse with clinically overt disease in the liver or abdominal cavity. Encouraging results have been obtained with an autologous tumor vaccine as an adjuvant therapy following tumor resection by demonstrating an increase in disease free and total survival (Hoover et al., J Clin Oncol 11:390, 1993). These findings combined with the demonstration of enhanced anti-tumor immunity following tumor immunizations with cells genetically modified to express IL-2 in several animal tumor systems provide the rationale for using IL-2 gene transfer in our study. Patients will receive immunizations with increasing doses of IL-2 transduced fibroblasts. The patients will be monitored for toxicity, anti-tumor responses and the induction of anti-tumor immunity. The results of the Phase I trial should permit an assessment of the safety of this form of cytokine gene transfer and provide initial data to evaluate the potential utility of IL-2 gene therapy with a mixture of autologous transduced fibroblasts and irradiated tumor cells.

NON-TECHNICAL ABSTRACT

The overall objective of this study is to perform a clinical trial in colon carcinoma patients comprising immunizations with a mixture of irradiated skin cells genetically modified to express the gene for an immunostimulatory substance, termed IL-2, and irradiated tumor cells. IL-2 gene transfer has resulted in significant anti-tumor immune responses in several animal tumor models. In these studies, the transfer of IL-2 genes into tumor cells has reduced or abrogated tumor formation after implantation into animals. We have successfully induced anti-tumor immunity in an animal model of colorectal carcinoma by immunization with a mixture of irradiated tumor cells and IL-2 transduced skin cells. Immunization with a mixture of irradiated tumor cells and IL-2 modified cells induced anti-tumor immunity capable of rejecting a subsequent live tumor cell challenge. Repeated immunizations with a mixture of irradiated tumor cells and IL-2 modified cells abolished established, visible tumors in a subset of the treated animals. Colorectal carcinoma is one of the most common cancers in the United States and Europe with an annual incidence of greater than 150,000 in the U.S. Most patients are treated with tumor resection and do not have clinically detectable tumor following surgery. However, the majority of patients have microscopic metastases and eventually relapse with clinically overt disease in the liver or abdominal cavity. Encouraging results have been obtained with an autologous tumor vaccine as an additional therapy following tumor resection. Immunization with tumor preparations resulted in a significant increase in disease free and total survival (Hoover et al., J Clin Oncol 11:390, 1993). These findings combined with the demonstration of enhanced anti-tumor immunity following tumor immunizations with cells genetically modified to express IL-2 in several animal tumor systems provide the rationale for using IL-2 gene transfer in our study. Patients will receive immunizations with increasing doses of IL-2 modified skin cells. The patients will be monitored for toxicity, anti-tumor responses and the induction of anti-tumor immunity. The results of the Phase I trial should permit an assessment of the safety of this form of gene transfer and provide initial data to evaluate the potential utility of IL-2 gene therapy with a mixture of genetically modified skin cells and irradiated tumor cells.

CLINICAL PROTOCOL

Immunization of Colon Carcinoma Patients with Autologous Irradiated Tumor Cells and Fibroblasts Genetically Modified to Secrete Interleukin-2 (IL-2) : A Phase I Study

--10/93

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1.0 Objectives

1. Evaluate the safety of subcutaneous immunizations with a mixture of irradiated autologous fibroblasts genetically modified to express the gene for IL-2 and irradiated autologous tumor cells.

2. Examine the effects of these immunizations on tumor growth *in vivo*.

3. Assess whether the immunizations induce cellular or humoral anti-tumor immune responses.

2.0 Background/Rationale

2.1 Interleukin-2 Cancer Immunotherapy in Human Subjects

Recent advances in our understanding of the biology of the immune system have lead to the identification of important modulators of immune responses (1-3). These agents mediate many of the immune responses involved in anti-tumor immunity. Several of these cytokines have been produced by recombinant DNA methodology and evaluated for their anti-tumor effects. In experimental clinical trials, the administration of cytokines and related immunomodulators has resulted in objective tumor responses in patients with various types of neoplasms (4-7).

Interleukin-2 (IL-2) is an important cytokine in the generation of anti-tumor immunity (4). In response to tumor antigens, a subset of lymphocytes termed helper T-cells secrete small quantities of IL-2. This IL-2 acts locally at the site of tumor antigen stimulation to activate cytotoxic T-cells and natural killer cells which mediate systemic tumor cell destruction. Intravenous, intralymphatic or intralesional administration of IL-2 has resulted in clinically significant responses in some cancer patients (4-6). However, severe toxicities (hypotension and edema) limit the dose and efficacy of intravenous and intralymphatic IL-2 administration (5,7). The toxicity of systemically administered cytokines is not surprising since these agents mediate local cellular interactions and they are normally secreted in only very small quantities.

To circumvent the toxicity of systemic IL-2 administration, several investigators have examined intralesional injection of IL-2. This approach eliminates the toxicity associated with systemic IL-2 administration (8,9). However, multiple intralesional injections are required to optimize therapeutic efficacy (8,9). These injections will be impractical for many patients, particularly when tumor sites are not accessible for direct injection without potential significant morbidity.

The aim of this study is to demonstrate the safety of a novel and more practical method of IL-2 cancer immunotherapy. In this approach, a patient's tumor cells are genetically modified to express and secrete IL-2. The genetically modified cells are then employed in subcutaneous immunizations to induce systemic anti-tumor immunity.

This approach provides the benefit of local IL-2 administration and obviates the need for multiple intralesional injections. The amount of IL-2 secreted by the genetically modified cells is sufficient to induce anti-tumor immunity but is too low to produce systemic toxicity. In addition, the continuous expression of functional amounts of IL-2 may also produce greater augmentation of anti-tumor immune responses compared to that obtained by intermittent cytokine injections.

2.2 Cytokine Gene Therapy in Animal Tumor Models

Cytokine gene transfer has resulted in significant anti-tumor immune responses in several animal tumor models (10-13). In these studies, the transfer of cytokine genes into tumor cells has reduced or abrogated the tumorigenicity of the cells after implantation into syngeneic hosts. The transfer of genes for IL-2 (10,11), gamma interferon (IFN) (12), or IL-4 (13) significantly reduced or eliminated the growth of several different histological types of murine tumors. In the studies

employing IL-2 gene transfer, the treated animals also developed systemic anti-tumor immunity and were protected against subsequent tumor challenges with unmodified parental tumor (10,11). Similar inhibition of tumor growth and protective immunity were also demonstrated when immunizations were performed with a mixture of unmodified parental tumor cells and genetically modified tumor cells engineered to express the IL-2 gene. No toxicity associated with expression of the cytokine transgenes was reported in these animal tumor studies (10-13).

We have successfully induced anti-tumor immunity in a model of colorectal carcinoma by immunization with a mixture of irradiated tumor cells and IL-2 transduced fibroblasts. Immunization with a mixture of irradiated tumor cells and IL-2 transduced cells induced systemic anti-tumor immunity capable of rejecting a subsequent live tumor cell challenge. Similar inhibition of tumor implantation and the induction of systemic anti-tumor immunity with tumor cells genetically modified to express IL-2 were demonstrated by Fearon et al (10) in the same colorectal carcinoma model utilized in our studies and by Gansbacher and co-workers (11) in a murine model of fibrosarcoma. Our study extends these observations by further demonstrating the ability of IL-2 gene therapy to eradicate established tumors. Repeated immunizations with a mixture of irradiated tumor cells and IL-2 transduced cells abolished visible tumors in a subset of the treated animals. The results of these pre-clinical animal studies are described in detail in Appendix 12.6.

2.3 Immunotherapy of Colon Carcinoma

Colorectal carcinoma is one of the most common cancers in the United States and Europe with an annual incidence of greater than 150,000 in the U.S. Most patients are treated with tumor resection and do not have clinically detectable tumor following surgery. However, the majority of patients have microscopic metastases and eventually relapse with clinically overt disease in the liver or abdominal cavity. The large number of patients and the small tumor burden following surgical resection makes colorectal carcinoma an attractive candidate for adjuvant immunotherapy trials. It is generally acknowledged that immunotherapies are likely to be most effective when the tumor burden is low. In this regard, the immunomodulator Levamisole is currently approved for the treatment of patients with Duke's C tumors (metastases to abdominal lymph nodes). In addition, encouraging results have been obtained with an autologous tumor vaccine as an adjuvant therapy following tumor resection (14). In this study, immunization with autologous tumor preparations and the adjuvant BCG resulted in a significant increase in disease free and total survival (14).

2.4 Gene Transfer into Human Subjects

The methods we will utilize to transfer cytokine genes into cells are virtually identical to those employed in previously approved human gene transfer studies in which tumor infiltrating lymphocytes (TILs) were modified by retroviral gene mediated transduction and administered to cancer patients (15). In this earlier Phase I study of retroviral mediated gene transfer, TILs were genetically modified to express the neomycin phosphotransferase (neo^R) gene. Following intravenous infusion, polymerase chain reaction analysis consistently demonstrated the presence of genetically modified cells in the circulation for as long as two months after administration. No infectious retroviruses were identified in these patients and no side effects due to gene transfer were noted in any patients (15). Additional Phase I clinical protocols employing similar retroviral gene transfer methods have recently been approved by the Recombinant DNA Advisory Committee (RAC). Our Phase I study of human cancer patients will utilize the same gene transfer methods and safety testing procedures employed in these previously approved investigations.

3.0 Treatment And Monitoring Schema (Revised)

The ideal treatment and monitoring schedules are summarized in Figures 5 and 6. After each injection, patients will be contacted daily for 3 days to determine if the injection site is swollen or red. If a history of any erythema or induration is elicited, then the patient will be examined within 24 hours. All subjects will be examined 14 days after each immunization. Physical examinations and laboratory evaluations will be performed every two weeks during the first 8 weeks of the study, once a month for three months and every 3 months for one year after the completion of therapy and at least yearly thereafter.

Figure 5 Treatment and Monitoring Summary

Day - 60	Skin biopsies Genetic modification of fibroblasts
Days + 0,14,28	Immunization with irradiated transduced tumor cells

Figure 2

TREATMENT AND MONITORING SCHEDULES

Treatment or Evaluation	Day of Study																	
	0	1	2	3	7	14	15	16	17	21	28	29	30	31	35	42	60	90
Immunization	x					x					x							
History/Physical	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Laboratory																		
CBC	x				x	x				x	x				x	x	x	x
PT, PTT	x				x	x				x	x				x	x	x	x
Chem 20	x				x	x				x	x				x	x	x	x
CH50 C3, C4	x				x	x				x	x				x	x	x	x
UA	x				x	x				x	x				x	x	x	x
Viral/PCR Assays																		
10cc Green Top	x				x	x				x	x				x	x	x	x
Serum IL-2 Humoral Immunity																		
10 cc Red Top	x				x	x				x	x				x	x	x	x
Immunophenotyping Cellular Immunity Skin Testing																		
50 cc Green Top	x				x	x				x	x				x	x	x	x

4.0 Patient Selection and Registration

4.1 Eligibility

4.1.1 Age 18 years or older with no significatnt medical problems. Nonpregnant females.

4.1.2 Metastatic colon carcinoma with measurable disease on enhanced CT or MRI. Imaging studies must be performed within two weeks of starting treatment.

4.1.3 Patients must have failed or refused standard therapy. Patients receiving prior treatment, including chemotherapy must wait 4 weeks before starting treatment.

4.1.4 Expected survival must be at least 3 months.

4.1.5 Karnofsky performance of $\geq 60\%$.

4.1.6 Baseline hematology and chemistry studies: hemoglobin > 9.9 gm/dl, total granulocyte count > 1,999/mm^3, platelet count > 124,000/mm^3, BUN < 30mg/dl, creatinine < 2mg/dl, alkaline phosphatase and SGOT < 2x normal upper limit of normal, amylase, lipase < 1.5x upper limit of normal. These studies must be performed within a week prior to starting therapy.

4.1.7 Informed Consent: All patients must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedures to be performed, the experimental nature of the therapy, alternatives, potential benefits, side effects, risks and discomforts. Human protection committee approval of this protocol and consent form is required.

4.2 Registration

4.2.1 All patients must be enrolled onto study through the San Diego Regional Cancer Center prior to starting therapy. To register a patient, contact Colette Carson, R.N. , Clinical Trials Registrar (619-450-5990). Please provide the following information:

Physcian's name and phone number
Institution's name
Patient's name and hospital unit number
Patient's date of birth, sex, race, height and weight
Previous treatment (surgery, radiation therapy, chemotherapy) with dates
(must be > 6 weeks from enrollment or patient's tumor must be progressing such that waiting 6 weeks is inadvisable)
Date and results of the following tests:
hemoglobin (must be > 9.9/gm/dl)
platelet count (must be > 124,000/mm^3)
BUN (must be < 30mg/dl)
creatinine (must be < 2mg/dl)
alkaline phosphatase, SGOT (must be < 2x normal upper limit)
amylase, lipase (must be < 1.5x normal upper limit)

4.2.2 The registrar will review the eligibilty checklist to verify patient eligibility. If the patient meets all criteria, the patient will be registered onto this study.

4.2.3 The registrar will remind the person registering the new patient of the expectations concerning submission of data and laboratory specimens.

5.0 Therapeutic Modalities

5.1 Preparation and Characterization of Biological Agents

5.1.1 Genetically Modified Autologous Tumor Cells and Fibroblasts

Skin punch biopsies and clinically indicated colon tumor resections will be obtained under sterile conditions. The tumor tissue will be minced and prepared as a single cell suspension for cryopreservation as described for the large prospective, randomized trial of adjuvant active immunotherapy of colon cancer with autologous tumor (14). The skin biopsy tissues will be minced and placed in DMEM media containing 10% fetal calf serum to establish growth of skin fibroblasts in culture. The cultured cells will be genetically modified by retroviral mediated gene transfer to express the IL-2 gene product.

5.1.2 Retroviral Vector

The retroviral vector utilized in this study is derived from a murine retrovirus similar to the vectors employed by a number of investigators for *in vitro* and *in vivo* studies including recently approved investigations with human subjects (15). The cytokine vector used in this investigation (LNCX/IL-2) contains a recombinant human IL-2 cDNA under the control of an internal CMV promoter. Before utilization in human studies, the transduced cells will be certified to be free of contaminating replication competent virus and other adventitious agents by criteria recommended by the FDA. The assays to be performed will include the following:

Sterility testing by bacterial and fungal culture

Mycoplasma testing by Gen-Probe kit assay

Replication competent retrovirus testing by PG-4 S+L- assay

Additional safety and quality assurance assays will be performed as described in Appendix 12.5 (Laboratory Procedures and Safety Testing)

5.1.3 Transduction Protocol

The cultured skin fibroblasts will be incubated with conditioned supernatant from the producer cell culture transfected with the retroviral vector plasmid as described (16). The fibroblasts will be washed and then grown in culture media containing G418, (a neomycin analogue) to select for transduced cells expressing the neo^R gene. G418-resistant cells will be stored at -70° C until required. Prior to immunization, the G418-resistant cells will be tested for expression of the IL-2 gene by measurements of the concentration of IL-2 in the culture supernatant by an enzyme-linked immunoabsorbent assay (ELISA).

5.1.4 Preparation of Irradiated Cells

After transduction and expansion, the genetically modified cells will be carried in continuous culture until there are sufficient cells for use in therapeutic applications or for cyropreservation. The cyropreserved cells will be centrifuged and washed in DMEM media and then cryopreserved in a solution containing 10% dimethyl sulphoxide and 50% fetal calf serum in DMEM media. The cells will be stored in liquid nitrogen until the time of administration. Non-transduced tumor cells utilized for immunizations will be treated with 10,000 rads as described for the adjuvant active immunotherapy trial of colon cancer with autologous tumor (14) and resuspend in lactated Ringer's solution prior to injection. Transduced fibroblasts will be irradiated with 3,000 rads to minimize the risk of chronic local inflammatory reactions at the site of immunization due to continued secretion of

IL-2. The dose of radiation to treat the fibroblasts (3,000 rads) was chosen following consultation with Drs. George Jones and Klaus Erikson of the UCSD Department of Radiobiology. This dose will result in the survival of less than one in 10^6 cells (data extrapolated from a published study on the radiation sensitivity of normal fibroblasts-Cornforth MN and Bedford JS Radiation Research 111:385,1987). Furthermore, in our animal studies with murine fibroblasts, this dose of radiation did not affect the efficacy of immunization with a mixture of tumor cells and irradiated, transduced fibroblasts.

5.2 Potential Toxicity

Local toxicity at the sites of immunization are not expected to occur. If severe inflammation occurs at the site of injection, this will be treated by either topical steroid therapy and/or surgical excision of the inflamed tissues as indicated.

Hypersensitivity reactions are unlikely to occur, since the injected material is comprised of minimally modified cells. Systemic toxicity related to IL-2 administration should not occur as the low levels of IL-2 secreted by the genetically modified cells should not significantly effect systemic IL-2 concentrations.

There are several theoretical toxicities related to the use of retroviral mediated gene transfer. These theoretical toxicities are not expected to occur as the retrovirus utilized for gene transfer has been modified to prevent these toxicities. The retroviral vector has been altered to prevent viral replication by the deletion of viral gag, pol and env genes. Hence, the IL-2 retroviral vector is replication defective and patients' cells are never exposed to a replication competent virus. Replication competent retrovirus may theoretically develop by recombination between the IL-2 vector and viral gene sequences in the packaging cell line utilized to produce the retroviral vector. This type of recombination has rarely been observed after extended culture. Hence, all retroviral vector supernatants used to infect patient cells will be screened for replication competent virus by standard assays (15). It is theoretically possible for the retroviral vector to insert the transgene into a proto-oncogene or tumor suppressor gene resulting in cellular transformation by insertional mutagenesis. The likelihood of this occurring is extremely small.

The neo^R gene transferred into the genetically modified tumor cells inactivates neomycin and related antibiotics such as amikacin. As the genetically modified cells will be injected subcutaneously, local expression of the neo^R gene is unlikely to effect treatment with these antibiotics at other sites. In addition, other more commonly used antibiotics such as gentamycin and tobramycin are not inactivated by the neo^R gene. Hence, the introduction of this gene into a local area of the patient is not expected to affect clinical management of infections.

5.3 Treatment of Toxicity

Local toxicity at the sites of cell administration will be treated with either topical steroids and/or surgical excision of the injection site as deemed appropriate. Hypersensitivity reactions may occur. Chills, fever and/or rash may be treated symptomatically with antipyretics and antihistamines. Patients should not be treated prophylactically.

Should arthralgias, lymphadenopathy or renal dysfunction occur, the investigators should be notified and treatment with corticosteroids and/or antihistamines may be instituted. Anaphylactoid type hypersensitivity reactions are not anticipated. However, should anaphylaxis occur, administration of epinephrine, fluids, steroids and cardiopulmonary support should be instituted as needed.

6.0 Treatment Plan

6.1 Location

Patients will be treated at Sharp Memorial Hospital, San Diego, CA.

6.2 Administration of Biological Agents

Subjects will receive at least three subcutaneous immunizations at least two weeks apart with a mixture of irradiated autologous fibroblasts genetically modified to express the gene for IL-2 and irradiated autologous tumor cells.

All subjects will be treated as outpatients. Vital signs will be checked prior to immunization. Subsequently, vital signs should be obtained qlh x 2. The patients should be examined qlh x 2 for inflammation at the injection site and for evidence of rash, wheezing or edema. Provided that there are no contraindications, subjects may be discharged 2 hours after drug administration. If discharge is contraindicated, patients will be followed in the hospital on a regular basis as deemed appropriate.

7.0 Therapy Modification

7.1 Dose Escalation

In a single patient with glioblastoma treated with IL-2 secreting tumor cells or fibroblasts, transient erythema at immunization sites was not observed until the IL-2 dose exceeded 100 units/24 hrs. In our animal model, this dose of IL-2 was effective in generating systemic anti-tumor immunity (see Appendix 12.6). Hence, we plan to initiate therapy with transduced fibroblasts that secrete this dose of IL-2. Our data indicate that the transduced fibroblasts will express approximatley 200 to 400 units of IL-2 per 10^6 cells per 24 hours. This will result in the administration of approximately 5 x 10^5 transduced cells for the initial immunizations depending upon the level of IL-2 expression measured *in vitro* per 10^6 cells over 24 hours. We plan to escalate the dose of IL-2 secreting cells, as follows:

	Approximate Units of IL-2	Approximate # of Transduced Cells
Cohort 1	100	5×10^5
Cohort 2	200	1×10^6
Cohort 3	400	2×10^6

The transduced fibroblasts will be mixed with 1 x 10^7 unmodified irradiated tumor cells for immunization. The dose of transduced cells will be escalated when 3 patients at each dosage level have been treated and followed for 1 month without $\geq$ grade 3 toxicity. Toxicity grades are listed in Appendix 12.1. If 3 patients at the same dosage level develop unacceptable treatment related toxicity ($\geq$ grade 3 toxicity), the study will continue at the previous dose level until a total of 15 patients have been treated.

Additional immunizations may be administered at approximately 2 week intervals, at the discretion of the investigators, as warranted by the demonstration of anti-tumor immune responses and improvement or stability in the patient's clinic status.

7.2 Maximum Tolerated Dose (MTD)

The maximum tolerated dose (MTD) is defined as that dose of IL-2 secreting cells which results in $\geq$ grade 3 toxicity or which causes a delay in day 14 immunization in $\geq$ 3/6 patients in any treatment cohort. Day 14 treatments must be delayed if any $\geq$ grade 2 toxicity has not reversed to $\leq$ grade 1.

8.0 Study Parameters

8.1 Pre-treatment Evaluations

The following pre-treatment evaluations will be performed (8.11-8.14):

8.1.1 History and Physical Examination

These evaluations will include a description and quantitation of disease activity.

8.1.2 Performance Status Assessment

See Appendix 12.2 for Karnofsky Performance Status assessment.

8.1.3 Pre-treatment Laboratory

CBC with differential, platelet count, PT, PTT, glucose, BUN, creatinine, electrolytes, SGOT, SGPT, LDH, alkaline phosphatase, bilirubin, uric acid, calcium, total protein , albumin, amylase and lipase.

Urinalysis

CH_{50}, C3 and C4 serum complement levels

Immunophenotyping of peripheral blood B cell and T cell subsets (if sufficient cells are available)

PCR of peripheral blood leukocytes for neo^R and viral env

Serum IL-2 concentration

Humoral tumor immunity - serum anti-tumor titers

Cellular tumor immunity - Cytotoxic T cell assays against autologous tumor cells. Delayed type-hypersensitivity (DTH) skin testing will be performed at the discretion of the investigators and depending upon the availability of tumor cells. Aliquots of 10^6 autologous tumor cells will be utilized for DTH skin testing. The cells will be drawn up in 0.2 ml of normal saline and injected intradermally. Induration will be measured at 24 and 28 hours. A medium ball point pen will be placed at 45^o angle to the patient's skin, and the injection site approached from each of 4 sides until resistance is encountered. The area thus circumscribed will be measured in 2 diameters with a caliper. Selected DTH sites may be biopsied for histological and immunohistochemical analyses to identify the nature of infiltrating immune effector cell populations.

8.1.4 Other Pretreatment Evaluations

Chest X-ray and other diagnostic studies including computerized tomography (CT), magnetic resonance imaging (MRI) or radionuclide scans will be performed to document and quantitate the extent of disease activity.

8.2 Follow-up Procedures

After each immunization, patients will be contacted daily for 3 days to determine if the injection site is swollen or red. If a history of any erythema or induration is elicited, then the patient will be examined within 24 hours. Otherwise, subjects will be examined one week after the first immunization, then at approximately 1 to 2 week intervals. At each visit, the patients will be advised to return to the hospital and to contact one of the study investigators should they develop inflammation at injection sites or experience fever, rash, arthralgias, edema or dyspnea.

At each visit, the pertinent medical history will be obtained and a physical examination will be performed. Laboratory tests described in Section 8.1 will be performed at the weekly and monthly visits.

Diagnostic studies including X-rays, CT, MRI or radionuclide scans will be performed to evaluate responses to therapy.

Should toxicity occur, appropriate therapeutic intervention will be instituted. Patients will be followed until toxicity resolves.

A summary of the treatment plan including the schedule for drug administration and required monitoring evaluations is provided in Section 3.0.

8.3 Off-Study Criteria

8.3.1 A patient will be considered off treatment once protocol therapy has been stopped. Reasons for discontinuation of treatment include:

Development of grade ≥ 3 toxicity
Patient's tumor fails to respond to treatment (progressive disease)
Patient has completed therapy
Patient refuses further therapy
Patient develops other medical problems unrelated to the study that preclude further therapy.

8.3.2 A patient will be considered off study in the event of disease progression, death or initiation of non-protocol treatment.

8.3.3 A patient who is off study will be off treatment; however, a patient who is off treatment is not necessarily off study.

8.3.4 Patients who are off treatment but not off study will continue to be followed for disease progression/response, late reports of toxicity and survival. Once a patient is off study because of disease progression or initiation of non-protocol treatment, the patient will be followed for survival purposes.

9.0 Evaluation Criteria

9.1 Adverse Drug Reaction Reporting

9.1.1 Reporting Requirements

The following adverse reactions must be reported to the study chair and the NCI, in addition, the institutions' IRB (Investigational Review Board) must be notified.

9.1.1.2 All life-threatening and lethal (grade 4 and 5) toxicity.

9.1.1.3 Grade 2 and grade 3 toxicity.

9.1.2 Types of Reports

9.1.2.1 Telephone Report. All life-threatening and lethal (grade 4 and 5) reactions must be reported to the study chair by telephone within 24 hours.

9.1.2.2 Written Report. An original NCI Adverse Drug Reaction form and 2 copies must be submitted to the study chair within 5 days. Immediately upon receipt the chair will forward the original report to NCI.

9.2 Response Criteria

9.2.1 Following the first two cycles of treatment, an evaluation of response will be performed. Tumor size will be calculated by measuring the tumor's largest cross sectional area as measured by the greatest cross sectional diameter times the greatest diameter perpendicular to it on enhanced CT or MRI. Technical parameters for the evaluation scan will be identical to those used for the baseline scan.

9.2.2 A physical examination will be performed and compared with the pretreatment examination.

9.2.3 A Karnofsky performance will be recorded and compared with the pretreatment score.

9.2.4 Medications and their dosages, including steroids, and laboratory data will be recorded in accordance with the study parameters above.

9.2.5 Response will be defined as summarized below.

Complete Response (CR): Disappearance of all measurable or evaluable disease, signs, symptoms and biochemical changes related to the tumor, for > 4 weeks, during which time no new lesions may appear. At the time of suspected CR, re-evaluate completely, short of invasive procedures. If hepatic or BM involvement were documented histologically, rebiopsy is required.

Equivocal Complete Response (Equiv. CR): This rating will be assigned where there is a complete response, but examination remains abnormal in such a way as to preclude an unequivocal statement that the tumor has completely disappeared.

Partial Response (PR): This rating will be assigned where there is a reduction by at least 50% of the product of the two longest perpendicular diameters of the lesion(s). A bone response consisting of recalcification of lytic bone metastasis will be included as partial response providing there has been no disease progression elsewhere.

Minor Response (MR): This rating will be assigned where there is a reduction of less than 50% of the product of the two longest perpendicular diameters of the lesion(s), and either no change in or partial recalcification of all osteolytic lesions.

Objectively Stable (S): This rating will be assigned where there is less than 25% increase in the product of the two longest perpendicular diameters of the lesion(s).

Progressive Disease (P): This rating will be assigned where there is an increase of greater than or equal to 25% in the product of the two longest perpendicular diameters of the lesion(s) or the occurrence of a new lesion(s).

10.0 Data Submission

The following forms must be submitted to the study chair according to the following schedule:

Forms	Schedule
On-study Form	Within one week of registration
Treatment Form	Within one week of treatment
Follow-up Form	Every month while receiving protocol
Adverse Reaction Reporting Form	Within five days for reportable toxic events

11.0 BIBLIOGRAPHY

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7. Sarna G, Collins J, Figlin R, Robertson P, Altrock B, Abels R. A pilot study of intralymphatic interleukin-2. II Clinical and biological effects. J Biological Response Modifiers 9(l):81-86, 1990.

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CONSENT TO ACT AS A RESEARCH SUBJECT

Injection of Colon Carcinoma Patients with Autologous Irradiated Tumor Cells and Fibroblasts Genetically Modified to Secrete Interleukin-2 (IL-2) : A Phase I Study

I am being asked to participate in a research study. However, before I give my consent to be a volunteer, it is important that I read the following materials and ask as many questions as necessary to be sure that I understand what my participation will involve.

Investigator and Sponsor

Dr. Robert E. Sobol and his colleagues are conducting an experimental study to learn more about methods to transfer genes into cancer patients' cells that may enhance the body's immune responses against cancer. I have been asked to take part in this study because I have colon cancer.

Purpose Of The Study

My skin cells will be altered in the laboratory by having a new gene inserted to generate a substance, termed interleukin-2, which has been shown to stimulate anti-tumor immune responses in some cancer patients. The purpose of this study is to determine the safety of injecting patients with the genetically modified cells mixed with a sample of tumor cells.

What Is Experimental About This Study

The process of injecting cancer patients with genctically modified cells and their tumor cells is experimental.

Number of Subjects

Approximately 9 patients will be treated.

Duration Of Study and Procedures To Be Followed

If I agree to be in this study, the following will happen to me: A small skin biopsy will be obtained from my back or arms. Skin cells obtained from the biopsy will be altered in the laboratory by having a new gene inserted to generate a substance, termed interleukin-2, which has been shown to stimulate anti-tumor immune responses in some cancer patients. In addition, a portion of my colon tumor removed during surgery will be saved and mixed with the genetically modified skin cells. The tumor and skin cell mixture will be injected underneath my skin. The cells will have been treated with x-rays to prevent their ability to grow. These injections are similar in nature to those utilized for routine vaccinations. I will receive three of these injections at two week intervals. All injections will be administered to me as an outpatient. Following each injection, I will be observed for one

hour to identify any side-effects of treatment. Subsequently, I will be contacted daily for three days following each injection and I will come to the clinic to be examined if redness or swelling develops at the injection sites. I will also be examined every two weeks during the first 8 weeks of the study, once a month for three months and every 3 months for one year after the completion of therapy and at least yearly thereafter to identify any side-effects of treatment and to evaluate the effects of these injections on my cancer. Follow-up studies will also include blood tests at each visit. Approximately 100 to 200 cc (10-20 tablespoons) of blood will be obtained at these visits. Radiology studies such as x-rays or CT scans will be performed after the third injection.

Men and Women Who Can Have Children

I understand that both men and women who participate in this study must agree to to use an effective contraception method (such as condoms, the pill, diaphragm or intrauterine device) to prevent reproduction for the duration of the experimental treatment.

Because this is a new medication and it has not been given to pregnant women, it is not known how it would affect the unborn baby. If I am pregnant, breast feeding or think I might become pregnant, I can't be in this research study. If I am not pregnant and if I am using a method of birth control approved by my doctor, I can be in this research study. If I cannot have children because of menopause or surgical sterilization, I can be in the research study.

Medications

Before taking any drug other than the study injections, I should first discuss it with the study physician. This includes over-the-counter medications such as pain relievers and antacids.

I will tell my study physician or research assistant about the medications I am taking, including why and how often I take the medicine, and the strength of these medications.

I understand that I should not drive or operate complicated machinery if drowsiness or sedation results from any of my treatments.

Risks And Discomforts

Participation in this study may involve some added risks or discomforts. These include:

1. There may be some pain, bleeding or infection at the skin biopsy site. These potential complications are rarely severe. The biopsy site will be closed with one or two stitches which will be removed approximately 10 days after the biopsy.

2. There may be pain, itching or a rash at the injection sites. These complications will be treated with medications, should they occur. If these side-effects at the injection sites are severe and do not respond to medications it may be necessary to surgically remove the affected tissues at the injection sites. Surgical removal of this tissue may be associated with pain, bleeding, infection and scar formation.

3. I may experience an allergic reaction to the injected materials. Allergic reactions may include symptoms such as fever, chills, rash, itching and low blood pressure. In some patients, these allergic reactions may be so severe as to be life-threatening. These allergic reactions are not expected to occur and they will be treated in the event that they develop. Because this is a new and untried treatment, it is possible that other risks not mentioned and not presently known could occur.

4. The injections may induce immune responses against normal cells in the digestion system and cause gastritis or colitis. Immune responses directed against normal tissues are termed "autoimmune reactions". Other possible autoimmune reactions may include skin rashes and joint inflammation. These reactions will be treated with appropriate medications.

5. The research methods employed in this study utilize a virus to infect and transfer the interleukin-2 gene into the cells. No serious side-effects have been observed in limited human studies with this procedure. There are, however, some theoretical risks associated with these methods. While the virus utilized to transfer genes into my cells has been modified to prevent its ability to proliferate and infect other cells, it is theoretically possible for the virus to become infectious. However, this is not expected to occur. A blood test will be performed to determine whether an infectious virus has been generated. If an infectious virus is detected, I will be isolated in a hospital room or my home until the virus disappears from my blood.

6. The genetically modified cells will also contain a gene that produces a protein that inactivates certain antibiotics. These antibiotics are not commonly used in the treatment of human infections. In addition, as many other more commonly used antibiotics are not inactivated by this substance, the procedure is not expected to affect the therapy of bacterial infections that may occur during the course of my illness.

7. There may be some pain, bleeding, bruising or infection at the blood withdrawal sites in my arms. These potential complications are rarely severe.

Benefits Of The Study

There may be no direct benefit to me from this experimental therapy. It is possible that these treatments may enhance my body's immune response to my cancer. However, even if this occurs, this may have no beneficial effect on the course of my illness. However, from my participation in this study, the investigators may learn more about the role of the

body's immune responses against cancer and about the use of gene transfer methods. This information may prove useful in the therapy of cancer patients in the future.

Request for Autopsy

I have been informed that important information may be obtained from an autopsy in the event of my death at any time in the future. I give my permission for this evaluation.

Alternative Methods of Treatment

I understand that alternative experimental treatments may also be available or that I may be treated by supportive care only without experimental therapy.

Emergency, Side Effects, Illness or Questions About The Study

If I need medical treatment for any reason, I must contact my study physician or call the on-call physician at the following telephone numbers:

Study Physician: San Diego Regional Cancer Center: 619-450-5990

On Call Physician: Sharp Memorial Hospital: 619-541-3400

If I have any questions about this study, please call: Dr. Robert E. Sobol at 619-450-5990 between the hours of 8am - 4pm.

Compensation for Injury

The cost of treatment for injuries that result from participation in this research will be covered. I understand that no other form of compensation will be provided if I am injured. For further information about this, I may call the Human Subjects Committee Office at 619-450-5990.

Confidentiality

My research records will be kept confidential, but will be available for examination by qualified representatives of federal agencies.

Subject Cost/Payment For Participation

I will not receive any money for participating in this study.

Subject Rights And Study Withdrawal

Participation in research is entirely voluntary. I may refuse to participate or withdraw at any time without jeopardizing my future healthcare benefits. I understand that I am not giving up any of my legal rights by signing this consent form.

Participation in this study may be ended without my consent if:

- the investigator believes that it is in my best interest.
- I don't follow the study procedures.
- the sponsor stops the study or tells my doctor that I must stop the study.

It is important that I understand all of the important details about the study. If I have any questions that are not answered by these materials, I should contact the study physician for more information.

If I have any questions about my rights as a subject in a research study, I should contact the Institutional Review Committee Chairman through

Linda Bergen, Coordinator
Institutional Review Committee
(619) 450-5990

This research project has been reviewed by an institutional review board for the protection of the rights of human subjects in research studies, in accordance with federal regulations.

Signature And Acknowledgment

My signature below means that I have read the above information about **Injection of Colon Carcinoma Patients with Autologous Irradiated Tumor Cells and Fibroblasts Genetically Modified to Secrete Interleukin-2 (IL-2) : A Phase I Study** and I have had a chance to ask questions to help me understand what I will do in this study. I agree to be in the study and I have been told that I can withdraw from the study at any time. I have been given a copy of this agreement and a copy of the Experimental Subject's Bill of Rights. I have been told that by signing this consent form I am not giving up any of my legal rights.

_____ _____

SIGNATURE OF SUBJECT DATE

SIGNATURE OF WITNESS DATE

PHYSICIAN'S SIGNATURE DATE

11/93

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

CVs

NAME	POSITION TITLE
Robert E. Sobol, M.D.	Adjunct Associate Member

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Boston University, Boston, MA	B.A.	1974	Philosophy
Chicago Medical School, Chicago, IL	M.D.	1979	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

1979-1980	Internship, Internal Med, University of Southern California Medical Center, Los Angeles, California
1980-1982	Research Fellow in Hematology/Oncology, University of California, San Diego, San Diego, California
1982-1983	Assistant Research Immunologist, U.C.S.D. Cancer Center, University of California, San Diego, San Diego, CA
1983-1990	Associate Physician, University of California, U.C.S.D. Cancer Center, University of California, San Diego, San Diego, CA
1986-1989	Resident, Internal Medicine, Mercy Hospital, San Diego, California
1990-1992	Clinical Fellow in Hematology/Oncology, U.C.S.D. Cancer Center, University of California, San Diego, San Diego, CA
1991-Present	Adjunct Associate Member, San Diego Regional Cancer Center

Publications (Partial list of 65 publications)

1. Sobol RE, Dillman RO, Smith JD, et al: Phase I evaluation of murine monoclonal anti-melanoma antibody in man: Preliminary observations. Hybridomas in Cancer Diagnosis and Treatment. Mitchell MS, Oettgen HF (eds). Raven Press, New York, Prog Cancer Research Thor 21:199-206, 1982.
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Davey FR, Mick R, Nelson DA, MacCallum J, Sobol RE, et al: Morphologic and cytochemical characterization of adult lymphoid leukemia which express myeloid antigen. Leukemia, 2:420-426, 1988.

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Sobol RE, Fakhrai H, Gjerset R, Shawler DL, Royston I, et al: Cytokine Gene Therapy: Active Tumor Immunotherapy With Transduced Fibroblasts. Proceedings of the American Association for Cancer Research, 33:2957, 1992.

Fakhrai H, Shawler DL, Gjerset R, Royston I, Sobol RE: Immune mechanisms and importance of promoters in IL-2 gene therapy with transduced fibroblasts. Proceedings of the American Association for Cancer Research, 1993 (Submitted).

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Ivor Royston, M.D.	President and Scientific Director

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
George Washington Univ., Washington, DC			Pre-medicine
Johns Hopkins Univ., Baltimore, MD	B.A.	1967	Human Biology
Johns Hopkins Univ., Baltimore, MD	M.D.	1970	Medicine
Stanford University, Stanford, CA		1977	Medical Oncology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience

1970-71 Intern, Internal Medicine, Stanford University Hospital, Stanford, CA
1971-72 Resident in Internal Medicine, Stanford University Hospital, Stanford, CA
1972-73 Staff Associate, Division of Virology, Bureau of Biologics, NIH, Bethesda, MD
1973-75 Chief, Viral Oncology Section, Division of Virology, Bureau of Biologics, NIH, Bethesda, MD
1975-77 Post-doctoral Fellow, Division of Oncology, Department of Medicine, Stanford University Schoo of Medicine, Stanford, CA
1977-78 Staff Physician, Veterans Administration Medical Center, San Diego, CA
1977-82 Assistant Professor of Medicine, Division of Hematology/Oncology, University of California, San Diego, School of Medicine, La Jolla, CA
1978-81 Clinical Investigator, Veterans Administration Medical Center, San Diego, CA
1980-91 Immunology & Genetics Committee, Cancer and Leukemia Group B
1981-91 Vice Chairman, Immunology and Genetics Committee of Cancer and Leukemia Group B
1981-90 Director, Cell Surface Marker Laboratory, UCSD Cancer Center, San Diego
1981-84 Chief, Medical Oncology, San Diego Veterans Administration Medical Center, San Diego, CA
1982-90 Associate Professor of Medicine, Division of Hematology/Oncology, UCSD School of Medicine
1982-85 Consulting member of the Biological Response Modifiers Program Decision Network Committee, Division of Cancer Treatment, National Cancer Institute/National Institutes of Health
1983-88 Member, Clinical Cancer Program Project Review Committee, NCI/NIH
1984-90 Director, Clinical Immunology Program, UCSD Cancer Center
1986-88 Member, UCSD Biotechnology Transfer Advisory Committee
1986-Pres. Co-Director, International Conference on Monoclonal Antibody Immunoconjugates for Cancer
1989-90 Member, Long Range Planning Committee, UCSD Cancer Center
1990-Pres. President & Scientific Director, San Diego Regional Cancer Center, San Diego, CA
1990-Pres. Adjunct Professor of Medicine, UCSD School of Medicine, San Diego, CA
1992-Pres. Co-Director, International Gene Therapy of Cancer Conference
1992-Pres. Member, Department of Veterans Affairs Merit Review Board for Oncology
1992-Pres. Member, Molecular Biology of Cancer Editorial Board
1992-Pres. Member, American Association for Cancer Research Exhibits Committee
1992-Pres. Chairman, Special Review Committee-Clinical Trials of Cancer with BRMs, NCI-NIH
1993-Pres. Member, Biotechnology Advisory Committee, for Congresswoman Lynn Schenk

Partial list from a bibliography of 113 publications:

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9312-061 Schuening, Friedrich; Fred Hutchinson Cancer Research Center, Seattle, Washington; *Retrovirus-Mediated Transfer of the cDNA for Human Glucocerebrosidase into Peripheral Blood Repopulating Cells of Patients with Gaucher's Disease.*

Date of RAC Approval: 12-2-93
Date of NIH Approval: 11-15-94

APPENDIX E: Scientific Abstract

Gaucher's disease is the most frequent sphingolipid storage disorder and affects about 30,000 patients in the United States. It is an autosomal recessive hereditary deficiency of a lysosomal enzyme, glucocerebrosidase, required for glycolipid degradation. The absence of a functional glucocerebrosidase leads to accumulation of glucosylcerebroside in macrophages throughout the reticular endothelial system. This lipid storage typically leads to hepatosplenomegaly, hypersplenism and lytic bone lesions.

The purpose of this study is to investigate whether ex vivo retrovirus-mediated transfer of the cDNA for human glucocerebrosidase into peripheral blood repopulating cells of patients with Type 1 Gaucher's disease followed by infusion of the transduced cells into the patient is safe and able to improve or cure the disease. Peripheral blood repopulating cells will be mobilized by treatment of the patient with recombinant human granulocyte colony-stimulating factor collected by repeated leukapheresis and selected for CD34 positive cells by avidin-biotin-immunoadsorption. CD34 positive cells will then be transduced ex vivo over a 5-day period in a long-term marrow culture system containing medium with retrovirus supernatant. After transduction, cells will be infused into the patient without myeloablative treatment. Collection, transduction and infusion of transduced peripheral blood cells will be repeated two times at 2-month intervals to increase the amount of transduced repopulating cells transplanted.

The primary endpoint of this study is to examine the safety of infusing peripheral blood repopulating cells transduced with the human glucocerebrosidase cDNA. The persistence and expression of the glucocerebrosidase gene in hematopoietic cells after infusion will also be studied.

APPENDIX F: Non-Technical Description of Experiment

Gaucher's disease is an inherited disease which causes a fatty substance to accumulate in bone marrow derived cells called macrophages. This accumulation of fat is due to the abnormality in an enzyme called glucocerebrosidase and results in many health problems. Currently, two forms of treatment are available. One consists of repeated intravenous injections of normal enzyme. This treatment needs to be continued life-long and is very expensive. The second form of treatment is transplantation of bone marrow from a suitable healthy donor. This treatment has a risk of transplant-related mortality. Furthermore, only about ¼ of the patients have a suitable marrow donor.

A possible new treatment is called gene therapy. A normal glucocerebrosidase gene is transferred into peripheral blood stem cells of a patient with Gaucher's disease. Stem cells are special cells which are able to produce platelets, red blood cells and white blood cells, including macrophages. The normal glucocerebrosidase gene is transferred into stem cells using modified viruses called retrovirus vectors which act as carriers of the gene. Once the gene is transferred into the stem cell, all the cells produced by the stem cell will contain the glucocerebrosidase gene, including the macrophages. This treatment, if successful, will provide the patient life-long with macrophages which contain a functioning glucocerebrosidase enzyme and are, therefore, not accumulating anymore fat and thereby causing disease. The retrovirus vectors used as carriers for the gene are altered such that they can transfer the gene to the stem cell but they cannot cause an infection.

Patients entered into this study will be treated for 4 days with a hormone-like drug called G-CSF, which increases the number of stem cells in the blood. On days 4 and 5 of G-CSF treatment, the patient's peripheral blood cells will be obtained by leukapheresis (peripheral blood cell collections with special machines). Cells will be enriched for stem cells using a device which selectively retains cells carrying the CD34 marker which is also on the surface of stem cells. The stem cell rich fraction will then be exposed to retrovirus vectors which transfer the glucocerebrosidase gene into the stem cells. The cells with the new gene will then be infused into the patient by injection into a blood vessel. This procedure of peripheral blood cell harvest, genetic alteration, and infusion into the patient will be repeated two more times at 2-month intervals. The patients will be observed for any possible side effect that may result from this treatment. Samples of blood and marrow cells will be obtained periodically and analyzed for the number of cells that contain the new gene and for the amount of glucocerebrosidase produced by these cells.

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#838.0

FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE, DIVISION OF ONCOLOGY
(10/05/93)

1. **Retrovirus-Mediated Transfer of the cDNA for Human Glucocerebrosidase into Peripheral Blood Repopulating Cells of Patients with Gaucher's Disease.**

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2. Introduction

The purpose of this study is to investigate whether *ex vivo* retrovirus-mediated transfer of the cDNA for human glucocerebrosidase (GC) into peripheral blood repopulating cells of patients with Type 1 Gaucher's disease followed by infusion of the transduced cells into the patient is safe and able to improve or cure the disease. Peripheral blood repopulating cells (PBRC) will be mobilized by treatment of the patient with recombinant human granulocyte colony stimulating factor (rhG-CSF), collected by repeated leukapheresis and selected for CD34 positive cells by avidin-biotin-immunoadsorption. CD34 positive cells will then be transduced *ex vivo* over a 5-day period in a long-term marrow culture system containing medium with retrovirus supernatant. After transduction, cells will be infused into the patient without myeloablative treatment. Collection, transduction and infusion of transduced peripheral blood cells will be repeated two times at 2 month intervals to increase the amount of transduced repopulating cells transplanted.

3. Background

A. Pathophysiology of Gaucher's Disease

Gaucher's disease, the most frequent sphingolipid storage disorder, is an autosomal recessive hereditary deficiency of a lysosomal enzyme required for glycolipid degradation (1-3). The absence of a functional glucocerebrosidase (or beta glucosidase or glucosylceramidase), which cleaves the glucosyl group from the ceramide, leads to accumulation of this glucosylcerebroside in macrophages throughout the reticular endothelial system (RES) (4). This lipid storage typically leads to hepatosplenomegaly, hypersplenism and lytic bone lesions. Incidence, age of onset and clinical course differentiate three major types of Gaucher's disease: the chronic adult type (type 1) that usually spares the CNS, the neuronopathic varieties of the acute infantile type 2 and the subacute type 3 disease. In contrast to type 1, types 2 and 3 manifest in infancy and early childhood with a rapidly deteriorating, eventually fatal course. While types 2 and 3 occur sporadically and are extremely rare, the non-neuronopathic type 1 has an approximate frequency of the heterozygous state of 1 in 37 in the Ashkenazi Jewish

population leading to an estimated birth frequency of 1 in 1400. There are about 30,000 patients in the United States with type 1 Gaucher's disease. While more than 25 mutations of the GC gene have been described, five specific mutations at nucleotide 1226, 84, 1448, 1297 and in intron 2 account for more than 97.5% of all cases in this population.

Clinical presentation of type 1 Gaucher's disease has a later onset with the average beginning of symptomatic disease at 25 years of age. The most common initial acute clinical presentations are bleeding manifestations resulting from increased thrombocyte sequestration in the enlarged spleen. Hepatic enlargement may become hemodynamically relevant leading to liver fibrosis and portal shunting with the development of varices and shunting through the lung. The most common manifestations of Gaucher's disease in the bone are lytic widening of the distal femurs, avascular necrosis of the femoral head, widening of the humeri, sclerosis and infarcts of the long bones and joint surfaces and other types of lytic lesions. Excruciating pain is the most troublesome of Gaucher's disease bone manifestations. It can occur anywhere in the skeleton, can be very severe and last for weeks or months. Compression fractures of the spinal vertebrae may occur. While ongoing organomegaly can be symptomatic in the form of abdominal discomfort and pain, liver failure with subsequent bleeding complications, and bone problems are the major cause of chronic morbidity in type 1 Gaucher's disease patients.

The diagnosis of type 1 Gaucher's disease is suspected based on genetic descent and presenting symptoms. It is established by demonstration of leukocyte glucocerebrosidase deficiency. Most patients have readily demonstrable Gaucher's cells in their bone marrow biopsy. The presence of these glucocerebroside-laden macrophages with characteristic striatae, inclusion bodies, an eccentrically located nucleus and a PAS-positive cytoplasm is a sign of disease activity. Therapy options in type 1 Gaucher's disease can be divided into symptomatic and curative approaches. Symptomatic treatments include those directed toward improvement of hematologic and bony manifestations. Thrombocytopenia and leukopenias, as well as other symptoms of organomegaly improve after splenectomy, however, this treatment has been associated with accelerated progression of bone disease by removing the major storage organ for the accumulating glucocerebroside. Bone lesions can be treated with orthopedic surgery such as hip joint replacements and pain therapy. None of these options have any influence on the underlying disease. In order to limit the disease progression and reverse some of its manifestations, a source of macrophage glucocerebrosidase activity must be provided. Two curative options have been clinically used to date. These include marrow transplantation and exogenous enzyme supplementation. Transplantation of marrow from an HLA-identical sibling has been demonstrated to repopulate the recipient RES with normal macrophages and led to the disappearance of Gaucher's cells in the RES, as well as reversing the organomegaly (5-9). However, a matched family donor is available for only about 25% of patients. Furthermore, bone marrow transplantation has a procedure-related mortality that may not be a justifiable risk in patients with chronic or slowly progressing non-neuronopathic Gaucher's disease. Enzyme replacement therapy with macrophage targeted glucocerebrosidase has been recently feasible (10-13). Through a process of large-scale purification and sequential deglycosylation of the native enzyme, the resulting alglucerase (ceredase) is taken up

after binding to the α-mannosyl-receptor on macrophage plasma membranes. Initial studies demonstrate that alglucerase therapy is capable of reversing glucocerebroside storage in the patient's organs. This safe and effective therapy is extremely expensive with the annual cost ranging between $100,000-$400,000 per patient depending on the treatment regimen and the patient's weight and is, for this reason, not an option for many patients. Therefore alternative treatment forms are necessary. One possible treatment modality is *ex vivo* retrovirus-mediated transfer of the human glucocerebrosidase cDNA into hematopoietic long-term repopulating cells of patients with Gaucher's disease followed by infusion of the transduced cells into the patient.

B. Retrovirus-mediated transduction of the human glucocerebrosidase cDNA into murine long-term repopulating marrow cells

Several investigators have demonstrated efficient transduction of the human glucocerebrosidase (GC) cDNA into a substantial fraction of murine long-term repopulating marrow cells using retrovirus vectors (14-17). Long-term reconstituted mice analyzed up to 8 months after transplantation of transduced marrow contained the intact provirus in marrow, spleen and thymus cells at greater than 1 copy per cell, indicating effective transduction of long-term repopulating hematopoietic cells. When marrow from the primary recipient mice was transplanted into secondary recipient animals, the intact provirus genome was detected in marrow, spleen, thymus and macrophages 4 months after secondary transplantation. Human GC mRNA was detected in macrophages as well as other hematopoietic cells of the animals. The levels of human GC enzyme activity in macrophages from marrow and spleen were equal to or greater than the endogenous mouse GC activity. Human GC could be precipitated with an antibody specific for human GC. Immunohistochemical analysis detected the human GC protein in 81% of the macrophages from the transplanted mice. These data indicate that murine long-term repopulating marrow cells can be efficiently transduced by retrovirus vectors containing the human GC cDNA and that high levels of human GC activity comparable with endogenous mouse activity can be observed in the majority of macrophages from these long-term reconstituted mice.

C. Retrovirus-mediated transduction of the neomycinphosphotransferase (*neo*) and the human adenosine deaminase (ADA) gene into canine long-term repopulating marrow cells.

Gene transduction into hematopoietic repopulating cells and long-term gene expression in large random bred animals has proven to be more difficult to achieve than in mice. One major difference between the experimental models is that the number of marrow donors, and therefore marrow cells, is basically unlimited in the inbred murine model, while in random bred animals, marrow is only available from one animal, the marrow recipient. This would also be the case in human applications. The amount of marrow cells available for vector transduction is therefore limited.

We have established a model for studying retrovirus mediated gene transduction into canine hematopoietic progenitor cells. The dog serves as a preclinical model to develop procedures which allow gene transduction into hematopoietic repopulating cells and to test the safety of these procedures before application of the techniques to humans. Two different methods have been used to transduce canine long-term repopulating marrow cells (18). The first method consisted of 24 hours cocultivation of marrow cells on

vector-producing packaging cells followed by incubation of marrow cells in a vector-containing long-term marrow culture system for 4 days. Transduced autologous marrow was infused into otherwise lethally irradiated (9.2 Gy TBI) dogs, and reconstituted marrow was examined for the presence and expression of retrovirus vectors. Six dogs received 0.4 - 1.0 x 10^8 marrow cells/kg transduced with retrovirus vectors which contained both the *neo* gene and the human ADA gene. Four of six dogs engrafted. Their marrows have shown 1-20% G418-resistant CFU-GM colonies intermittently for more than two years after transplantation (Table 1). G418-resistant colonies were not observed in marrows from control dogs.

Culture results were confirmed by polymerase chain reaction (PCR) demonstrating the presence of the *neo* gene in marrow cells so far more than three years after transplantation (Figures 1 and 2 and Table 2).

The *neo* gene was also found by PCR in peripheral blood and lymph node lymphocytes and peripheral blood granulocytes so far up to more than three years posttransplant (Figures 1 and 2 and Table 2). PCR analysis of those samples of marrow cells, peripheral blood granulocytes and peripheral blood and lymph node lymphocytes which had been shown to contain the *neo* gene also detected the gene for human ADA (Figure 3).

However, repeated analysis of peripheral blood and marrow cells for human ADA gene expression by starch gel electrophoresis was negative. This experiment demonstrates that transduction of marrow by cocultivation and long-term marrow culture leads to long-term persistence of the retrovirus vector in myeloid and lymphoid cells, strongly suggesting transduction of long-term repopulating stem cells. For currently unknown reasons, long-term expression of the *neo* gene but not the human ADA gene was observed.

In a different experimental approach, autologous marrow, obtained 7 days after cyclophosphamide treatment (40 mg/kg once i.v.) at the time of the peripheral blood neutrophil nadir, was cocultivated for 24 hours on vector-producing cells and reinfused (0.06 - 0.18 x 10^8 cells/kg) into four irradiated (9.2 Gy TBI) dogs. Two dogs engrafted and showed 1-10% G418-resistant CFU-GM colonies intermittently for so far more than two years, similar to the surviving dogs of the previous experiment (Tables 2 and 3). Marrow from control dogs did not show G418-resistant colonies.

PCR analysis of marrow cells, peripheral blood granulocytes and peripheral blood and lymph node lymphocytes obtained so far more than two years after marrow transplantation demonstrated the presence of the *neo* gene (Table 2). Similar to the previous results, no expression of human ADA was detected even though the human ADA gene was present in cells containing the *neo* gene (Figure 3). Thus, this experiment shows that cocultivation of marrow obtained from dogs after treatment with cyclophosphamide leads to long-term persistence of retrovirus vectors in myeloid and lymphoid cells, providing evidence for gene transduction into long-term repopulating stem cells. For unknown reasons, only the *neo* gene but not the human ADA gene was expressed long-term.

Dilution experiments with cells from the dogs indicate that the fraction of marrow cells,

peripheral blood granulocytes and peripheral blood and lymph node lymphocytes containing the *neo* gene is 0.1-10%, which is consistent with the percentage of G418-resistant CFU-GM observed. Peripheral blood samples of the dogs are free of helper virus and no long-term side effects from the transduction of the retrovirus have been observed so far with observation times between 2 and 4 years. One dog (C874) died due to metastatic prostate carcinoma 3 years after transplantation. DNA of multiple tumor samples was analyzed by PCR for the presence of retrovirus vector DNA. None of the samples contained vector DNA (Figures 4a and b), suggesting that the tumor developed independent of gene transduction, probably as a late side effect of total body irradiation (see detailed description on page 9). Intermittent gene expression is probably due to the small number of repopulating cells transduced, which produce committed progenitor cells intermittently for limited periods of time. These data demonstrate successful transduction of long-term repopulating marrow stem cells in a large random bred adult animal model without any long-term side effects.

D. Retrovirus-mediated *neo* gene transduction into canine long-term repopulating peripheral blood cells

Three dogs have been transplanted with transduced peripheral blood cells and untransduced marrow cells after 9.2 Gy TBI. After aspiration and cryopreservation of marrow, animals were treated with c-kit ligand for 8 days to mobilize peripheral blood repopulating cells. On day 7 of growth factor treatment, dogs underwent leukapheresis. Peripheral blood cells were selected for MHC class II antigen-positive cells by avidin-biotin immunoadsorption to enrich for early hematopoietic progenitor cells. Class II MHC-positive cells were then transduced by 24-hour cocultivation on PA317/LN packaging cells and 11-day incubation in a vector-containing long-term marrow culture system which was fed every other day with new vector-containing medium. After otherwise lethal irradiation with 9.2 Gy TBI, the dogs received between 0.05 and 0.12 x 10^8/kg transduced peripheral blood cells and, additionally, between 1.8 and 4.1 x 10^8/kg untransduced autologous bone marrow cells. The transduction rate of peripheral blood progenitor cells at the time of transplantation ranged from 2% to 18% as determined by G418-resistant CFU-GM colonies. All three dogs engrafted; however, one dog (D425) died from chronic canine distemper sclerosing encephalitis on day 84 after transplantation (see detailed description on page 9). Autopsy was performed and tissues from different organs were analyzed for *neo*-containing sequences by PCR. The results are shown in Figure 5. *Neo* was detected in peripheral blood granulocytes and bone marrow; all other tissues analyzed were negative. After hematopoietic reconstitution, marrow and peripheral blood cells were examined in the other two dogs for the presence and expression of the *neo* gene. So far, marrows have shown between 1 and 10% G418-resistant CFU-GM colonies for up to 48 weeks after transplantation (Table 4). The presence of the *neo* gene in marrow, peripheral blood granulocytes and lymphocytes continues to be demonstrated by PCR for up to 60 weeks after transplant (Figures 6-8 and Table 5). These data suggest transduction of peripheral blood-derived long-term repopulating cells. The two surviving dogs remained healthy and free of any side effects of the transduction procedure. Both dogs have been repeatedly tested for helper virus and none has been detected.

E. Retrovirus-mediated transduction of the human glucocerebrosidase gene into canine

long-term repopulating hematopoietic cells

Four dogs have been transplanted with peripheral blood and/or marrow cells transduced with retrovirus vectors containing the human glucocerebrosidase gene. Animals were treated with c-kit ligand for 7 days to mobilize peripheral blood repopulating cells and to stimulate early marrow progenitor cells to divide. On days 5 and 6 of growth factor treatment, dogs underwent leukapheresis. Peripheral blood cells were incubated for 7 days in a vector-containing long-term marrow culture system which was fed daily with retrovirus-containing medium. On day 7 of growth factor treatment, 5 days before transplantation, marrow was aspirated from all four legs. Cells were transduced either by 24-hour cocultivation on vector-producing packaging cells followed by 4-day incubation in a vector-containing long-term marrow culture system or by 5-day vector-containing long-term marrow culture only. After myeloablative treatment with either 9.2 Gy TBI or 40 mg/kg of cyclophosphamide, dogs received between 0.12 and 0.26 x 10^8/kg transduced peripheral blood cells and/or between 0.93 and 1.2 x 10^8/kg transduced marrow cells. All four dogs engrafted. After hematopoietic reconstitution, peripheral blood granulocytes and lymphocytes were analyzed by PCR for the presence of the human glucocerebrosidase gene. The gene has been found up to 18 weeks after transplantation in both myeloid and lymphoid cells to date. This suggests transduction of pluripotent long-term repopulating cells (Figure 9, Table 6). However, GC enzyme activity in peripheral blood cells was not increased as compared to activity in cells from control dogs. No obvious difference was found up to now regarding the efficiency of the different transduction methods used.

F. Retrovirus-mediated *neo* gene transduction into human G-CSF mobilized peripheral blood-derived CFU-GM

Eight patients were treated with G-CSF (16 µg/kg/day) for 7 days and underwent leukapheresis daily for 4 days starting on day 5 of growth factor treatment. In seven patients an aliquot of the peripheral blood cells collected was then selected for CD34-positive cells by avidin-biotin immunoadsorption and transduced by 24-hour cocultivation on PA317/LN packaging cells or by 5-day exposure to vector containing supernatant in a LTMC system. Between 10 and 51% G418-resistant CFU-GM colonies were observed (Table 7), similar to the results obtained with human marrow-derived CFU-GM (Table 8). This experiment suggests that human peripheral blood-derived CFU-GM can be transduced by retrovirus vectors similar to human marrow-derived CFU-GM. Transduction of CD34-positive peripheral blood cells appears to result in a higher transduction rate as compared to unselected cells.

G. Retrovirus-mediated transduction of the human glucocerebrosidase gene into human hematopoietic progenitor cells

Several investigators have demonstrated efficient transduction of the human glucocerebrosidase cDNA into a substantial fraction of human hematopoietic progenitor cells obtained from otherwise normal donors and from patients with Gaucher's disease (19-21). Between 35 and 45% transduced colony forming units (CFU) were observed when transducing marrow obtained from patients with Gaucher's disease. Enzyme activity of transduced Gaucher progenitor cells increased 3.5 to 6.2 fold as compared to progenitor cells transduced with a vector containing only the *neo* gene. Transduction of G-CSF mobilized CD34 positive peripheral blood cells obtained from normal donors with a GC vector resulted in a 1.8 fold increase in GC activity above endogenous levels.

Similar results were obtained in our laboratory when transducing G-CSF mobilized peripheral blood progenitor cells from normal donors (Figure 10). GC enzyme activity of cells transduced with GC containing vectors was increased 1.3 to 4.9 fold above endogenous enzyme activity of control cells transduced with *neo* containing vectors. Enrichment for CD34+ cells increased transduction efficiency 1.2 to 2.7 fold as compared to unselected controls.

CFU-GM colonies, obtained from normal peripheral blood cells which were transduced with GC containing vectors, were analyzed by PCR for the presence of the vector-derived GC cDNA. Three, five or ten colonies were picked from agar cultures and submitted to PCR amplification. PCR primers used were specific for a cDNA-fragment of the LTR-GC gene region of the vector and did not amplify the endogenous human GC gene in untransduced control colonies. Positive results in at least one of three colonies picked from the agar cultures indicate a transduction efficiency of at least 33% (Figure 11).

H. *Ex vivo neo* gene transduction into human marrow cells followed by autologous marrow transplantation

Brenner et al has transduced the *neo* gene into marrow cells harvested from children with acute myeloid leukemia in remission who were undergoing autologous marrow transplantation (22). The aim of this study was to determine whether relapse occurring after autologous marrow transplantation is due to residual leukemic cells in the transplant or only to residual disease in the patient. Patients have been followed so far for up to one year after autologous transplant. Two patients have relapsed and in both the blast cells contained the *neo* gene indicating that remission marrow can contribute to leukemic relapse. The second important result of these studies is the demonstration of the persistence of the *neo* gene in all hematopoietic lineages for so far up to one year after transplantation. These data strongly suggest that it is possible to transduce human long-term repopulating hematopoietic cells using retrovirus vectors.

I. Engraftment of hematopoietic repopulating cells in nonmyeloablated recipients

Using mouse or dog models, investigators have been able to demonstrate engraftment of long-term repopulating cells without prior myeloablative treatment of the recipient (23-27). Quesenberry et al (26) has shown long-term engraftment (up to 25 months posttransplant) of normal male donor marrow transfused into noncytoablated female mice when transplanting large numbers of donor marrow (40×10^6 cells daily i.v. for 5 consecutive days, which is the equivalent of marrow from 2 tibias and 2 femurs daily). When injecting 40×10^6 marrow cells once or 5×10^6 cells daily for 5 days, no engraftment was detected. Carter et al. (27) transplanted genetically (*neo* gene) marked canine long-term marrow culture cells into autologous recipients with (four dogs) or without (three dogs) myeloablative conditioning. Long-term persistence (up to 2 years posttransplant) of the *neo*-marked marrow cells was observed. There was no difference in the percentage of *neo*-marked cells present in dogs that received marrow ablative conditioning as compared to those receiving no conditioning. These data suggest that successful syngeneic or autologous transplantation of marrow cells is possible without the need to create space or open niches for the transplanted marrow by preceding cytoreductive treatment. Based on these experiments it is reasonable to expect

engraftment of autologous cells removed from a patient and treated *ex vivo* with retroviral vectors even if the patient's marrow is not ablated.

J. Safety issues of retrovirus-mediated gene transduction

The retrovirus vector LgGC and the PG13 packaging cell line (28) will be used for transduction of the CD34+ PBRC. The LgGC vector is derived from the LN vector. The vector LN is an improved version of the vector LNL6 (29), which has been studied extensively in rodent models and in non-human primates. It was approved for use in man in 1989. The results of the first *in vivo* human study using the vector LNL6 to transduce tumor-infiltrating lymphocytes have been reported (30). No side effects have been seen and no infectious virus has been detected in the patients.

1. The LgGC vector

The basis for the LgGC vector is the N2 vector (31), a derivative of Moloney murine leukemia virus in which the viral gag, pol, and env genes have been removed or truncated and the bacterial *neo* gene inserted. The retroviral packaging signal in N2, however, also contains the start codons for the gag protein of the parental helper virus, Pr65 gag and a larger glycosylated protein, g Pr85 gag, that initiates at a CTG codon upstream of and in frame with the ATG codon of Pr65. To prevent synthesis of viral proteins, the N2 vector was modified to LNL6 by inserting a stop codon in place of the Pr65 gag start codon and by replacing the upstream region of the vector with the homologous region from Moloney murine sarcoma virus, which is very similar to Moloney murine leukemia virus but does not make the glycosylated gag protein. However, undesirable generation of helper virus could still occur in these packaging cells by homologous recombination between viral sequences endogenous in the packaging cells and sequences in the constructed vectors, although this has not been observed so far. To prevent this possibility the vector LNL6 was modified to LN by removing all env sequences. The LgGC vector is derived from the LN vector by replacing the *neo* gene with human GC gene. The defective helper virus used to make PG13 packaging cells was truncated immediately after env; thus, the lack of homologous overlap between the vector LgGC and viral DNA in the PG13 packaging cells excludes the possibility of helper virus generation by homologous recombination and further increases the safety of the vector LgGC for use in humans.

2. Safety of retrovirus-mediated gene transduction into canine marrow or peripheral blood-derived repopulating cells

Nine of a total of 13 dogs engrafted after transplantation of transduced marrow or peripheral blood cells as described above. Eight of the nine dogs have been observed so far for more than 1 year after transplantation. Two dogs are alive more than 4 years after transplant, three more than 2 years, and two more than 1 year. One dog (C874) died 3 years after transplantation due to metastatic prostate carcinoma, and one dog (D425) died on day 84 from chronic canine distemper sclerosing encephalitis. Peripheral blood of the nine engrafted dogs was tested three times for replication-competent virus using the S^+L^- helper virus assay as described (32) (last test on 7-93). The S+L-assay is a sensitive assay for detection of replication-competent virus. It measures the ability of a virus sample to mobilize a retroviral vector from cells that contain but do not produce a vector. None of these tests showed the presence of replication-competent virus.

The dog (C874) which died 3 years after transplantation of transduced marrow cells showed at autopsy a prostatic tumor which infiltrated the urinary bladder and occluded the trigone of the bladder, thus leading to severe bilateral hydronephrosis and death secondary to uremia. On 1-3-92, 3 days before the death, serum creatinine was 10.2 mg/dl (normal 0.6-1.0 mg/dl) and serum urea nitrogen was 70 mg/dl (normal 6-30 mg/dl). Tumor metastases were found in liver, lungs and mesentery. Histology showed an adenocarcinoma of the prostate. Multiple samples from normal and tumor tissue were analyzed for the presence of retrovirus vector DNA by PCR amplification of *neo* gene sequences. Vector DNA was detected only in peripheral blood granulocytes (Figures 4a and b). None of the tumor samples contained retrovirus vector DNA.

Samples from normal and tumor tissue were also analyzed for the presence of replication-competent amphotropic proviral genomes by PCR amplification of amphotropic envelope gene sequences. No replication-competent proviral genome was detected. It is therefore unlikely that tumor development was related to gene transduction into transplanted marrow cells. Tumor development is most likely a late side effect of high-dose TBI used for myeloablation before infusion of transduced marrow cells. Observations in dogs have shown that TBI significantly increases the relative risk of developing a malignancy (33,34). Similar observations have been reported in Rhesus monkeys (35) and in human beings (36). Prostatic carcinoma is a rare malignancy in normal dogs. In our laboratory, 1 of 44 otherwise normal dogs which developed a malignancy, was diagnosed to have a prostatic carcinoma. After TBI 1 of 11 irradiated dogs which developed a malignant tumor showed an adenocarcinoma of the prostate.

One dog (D425) died on day 84 after transplantation of transduced peripheral blood cells from chronic canine distemper sclerosing encephalitis. On day 70, the dog was found to have convulsions and fever up to 104°F. Serum chemistry was normal except for a mildly increased glucose of 140 mg/dl (normal 31-109 mg/dl) and a slightly decreased phosphorus of 3.8 mg/dl (normal 4.0-9.2 mg/dl). The animal was treated empirically with a broad-spectrum antibiotic (Tazidime) as well as with phenytoin and diazepam for control of convulsions. The temperature decreased to 101°F and the convulsions disappeared. On day 81, the dog started biting, developed seizures, and became increasingly unaware of its surroundings. Serum chemistry on day 83 was completely normal including glucose and phosphorus. Due to its poor condition, the dog was euthanized on day 84 with pentobarbital. Autopsy did not show any macroscopic abnormalities. Histological examination of the brain revealed a subacute, multifocal sclerosing encephalitis which was most severe in the lateral and medial ventral frontal cortex, somewhat less severe in the inferior temporal cortex, and least severe in the thalamus. The lesions were characterized by marked lymphocytic perivascular cuffing, focal gliosis and an extensive invasion of the brain parenchyma with monoclonal cells, and foci of demyelination. Stains for bacteria, fungi, acid fast organisms and nocardia were negative. No viral inclusion bodies were seen in the brain sections; however, one to two inclusion bodies were seen in the renal pelvis epithelial cells. These lesions are compatible with chronic viral canine distemper. This form of distemper encephalitis represents a slow viral

infection similar to that associated with measles virus and subacute sclerosing panencephalitis in human beings. The disease is due to a paramyxovirus.

Multiple samples from different organs including brain were analyzed by PCR for the presence of retrovirus vector DNA or replication-competent amphotropic proviral genomes. *Neo* gene sequences were only detected in peripheral blood granulocytes and marrow cells (Figure 5). None of the samples contained amphotropic envelope gene sequences. It is therefore unlikely that the development of encephalitis was related to gene transduction into transplanted peripheral blood cells.

3. Safety of retrovirus-mediated gene transduction in Rhesus monkeys and humans

Five rhesus monkeys were exposed to replication-competent murine amphotropic retrovirus under various conditions to determine the potential clinical significance of an *in vivo* exposure to these viruses for primates (37). Retrovirus was infused intravenously into three normal animals and into one animal which was immunosuppressed by pretreatment with prednisone (5mg/kg/day) and cyclosporine A (10 mg/kg/day) for 26 days prior to virus infusion. Virus was cleared rapidly (within 15 minutes after the infusion) from the circulation and subsequent viremia has not been detected (mean follow-up of 27.4 months). A fifth monkey was immunosuppressed and received an intraperitoneal injection of replication-competent virus as well as a subcutaneous implant of virus-producing autologous fibroblasts. This animal developed viremia for two days post-inoculation. Replicating virus was recovered from peripheral blood mononuclear cells and lymph node cells for up to 22 days after virus-inoculation. Subsequent analysis has been negative for up to 5 years post-inoculation (38). No clinical illness was detected any time after virus exposure in the five animals studied. In a separate experiment, four monkeys received an otherwise lethal dose of total body irradiation followed by infusion of autologous marrow which had been exposed to a replication-defective retrovirus vector which was contaminated with replication-competent virus at a high titer (1×10^6 cfu/ml) (39). Animals were studied for up to 7 years after transplantation and remained alive and well (38). No replicating virus or amphotropic envelope sequences were detected in peripheral blood or marrow although antibodies directed against the p30 and gp70 viral antigens were found, indicating that the animals had been exposed to replication-competent retrovirus. These findings suggest that even during a time of severe immunosuppression after total body irradiation and bone marrow transplantation, primates can clear replication-competent murine amphotropic retroviruses. These results are in contrast to a recent observation that three of ten rhesus monkeys developed T-cell lymphomas 6 to 7 months after they had received marrow transduced with retrovirus vectors which were contaminated with replication-competent virus (40). Tumor cells contained the replication-competent virus, but lacked the retrovirus vector genome, suggesting that the spread of replication-competent virus through the regenerating hematopoietic tissue caused tumor development by insertional mutagenesis. These findings indicate the need for stringent screening of vector-producing packaging cells to exclude contamination with replication-competent virus.

In a clinical study (30), five patients were treated with infusions of tumor-infiltrating lymphocytes which had been marked with a *neo* gene containing retrovirus vector (LNL6) which is similar to the vector LgGC which will be used in this study. Extensive safety studies were carried out including S+L- assays for xenotropic and amphotropic infectious viruses, polymerase-chain-reaction analysis for the presence of the amphotropic helper virus 4070A envelope genes and reverse transcriptase assays. No safety hazards due to gene transduction were recognized including the absence of infectious virus in the infused lymphocytes and in the patients after infusion. So far, 21 humans have undergone gene-transfer procedures, and no side effects have been observed (41).

4. Recombination with human endogenous retrovirus sequences

Another theoretical concern is recombination of the retrovirus vector with human endogenous retrovirus sequences, leading to the production of a replication-competent human retrovirus. Again, the probability of this occurring appears small. No replication-competent human endogenous retrovirus has ever been isolated and all known sequences have deletions and frameshift mutations in the virus genes. The sequences defective in human endogenous retroviruses (namely gag, pol, and env), are the sequences deleted from the LgGC vector, so LgGC is unable to provide the needed sequences to restore function to human endogenous retrovirus sequences. Also, there is no homology known between the LgGC vector and the human retrovirus sequences, so that homologous recombination is not expected. In addition, variation in the structure of these human and murine virus sequences, such as tRNA binding sites, makes the likelihood of successful recombination extremely small.

These expectations have been borne out in preclinical and clinical practice. Recombination between MLV and human endogenous retrovirus sequences has not been detectable in primate cells *in vitro* or *in vivo*. This question was specifically addressed in the LNL6 TIL human gene transfer clinical trial. After retrovirus insertion, reverse transcriptase (RT) assays have never detected RT activity.

In summary, the gene transduction studies proposed in this protocol should produce minimal, if any, risk to the patient, no risk to other patients, and no risk to health care personnel.

Objectives

A. To investigate the efficiency and safety of transducing the human glucocerebrosidase cDNA into G-CSF mobilized, CD34 enriched peripheral blood repopulating cells from patients with Type 1 Gaucher's disease by retrovirus-mediated gene transfer.

B. To determine the extent of long-term persistence of transduced peripheral blood repopulating cells in Gaucher's disease patients not receiving prior myeloablation.

C. To investigate whether the human glucocerebrosidase cDNA is expressed sufficiently to improve the disease.

D. To determine whether repeated infusions of transduced peripheral blood repopulating cells increase the level of glucocerebrosidase activity in patients with Gaucher's disease.

5. Patient Selection

A. Inclusions

1. Patients ≥ 18 years of age with Type 1 Gaucher's disease as confirmed by
   a) glucocerebrosidase enzyme level determination, or
   b) DNA sequence analysis
2. Patients receiving glucocerebrosidase treatment must a) have had significant signs and symptoms of Gaucher's disease prior to treatment with glucocerebrosidase and b) have responded to this treatment in a measurable way in at least two of the following criteria:
   a) an increase in hemoglobin of 2 g/dl
   b) an increase in platelet count of 50%
   c) a decrease in spleen size of at least 25% or
      a decrease in liver size of at least 25%
   d) an improvement in MRI or X-ray of the bones
   e) a 50% decrease in non-tartrate inhibitable acid phosphatase
   f) a 50% decrease in angiotensin converting enzyme
3. Patients not receiving glucocerebrosidase treatment must have measurable clinical disease in form of at least two of the following signs and symptoms:
   a) splenomegaly or hepatomegaly
   b) anemia with a hemoglobulin < 11 g/dl
   c) thrombocytopenia with a platelet count < 90,000/mm^3
   d) disabling bone pain with degenerative changes on x-ray films
   e) pulmonary compromise with clubbing and hypoxemia ($Pa0_2$ < 70%)
   f) biopsy proven cirrhosis and elevation of hepatic parenchymal enzymes
   g) esophageal variceal bleeding
4. Adequate baseline organ function as assessed by the following laboratory values:
   a) creatinine clearance > 50 ml/min
   b) SGOT, SGPT and alkaline phosphatase ≤ 5 times upper limit of normal (ULN)
   c) PT and PTT < 1.5 times ULN
   d) Platelets ≥ 50,000/mm^3
   e) WBC > 2,000/mm^3
   f) absolute neutrophil count ≥ 1,000/mm^3
   g) serum amylase ≤ 1.5 times ULN
   h) bilirubin ≤ 2.5 mg/dl
   i) triglyceride < 400 mg/dl
5. Ability to give informed consent.
6. Normal cardiac function by history and physical exam.
7. Karnofsky Performance Status ≥ 60%.

B. Exclusions

1. Pregnant or lactating females; patients must practice adequate birth control; females of child-bearing potential must have a negative serum beta-HCG pregnancy test.
2. Patients with acute infections.

3. Patients who are HIV+.
4. Patients with cardiorespiratory instability.

6. Evaluation and Counseling of Patient

Patients will be referred for consideration of a gene therapy. The patient will be completely evaluated. The protocol will be discussed thoroughly with patient and family and all known risks to the patient described. The gene therapy procedure and alternative forms of therapy will be presented as objectively as possible and the risks and hazards of the procedure explained to the patient. Consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. A summary of the conference will be dictated for the medical record detailing what was covered.

7. Protocol Registration

Patients will be assigned to a protocol by the Ward Coordinator who will register the patient with the Registration Office (667-4728) between 8:30 am and 4:00 pm, Monday through Friday. At other times, registration should be obtained by calling the FHCRC's Nurses' Station at 667-5001 and ask for the registrar to be bellboyed.

8. Plan of Treatment

A. Treatment diagram

Marrow aspiration (OPD) and establishment of LTMC stromal layer, day 1
|
rhG-CSF, 16 μg/kg/day s.c., days 11-14
|
Central venous catheter placement, day 11 (OPD)
|
Peripheral blood cell (PBC) collections, day 14 and 15
|
Enrichment of PBC for CD34+ cells, day 15
|
Transduction of CD34+ PBC with GC-gene containing retrovirus vector supernatant, days 15-20
|
Analysis of pooled supernatants from transduced cells for replication competent virus and of a PBC sample for sterility and transduction efficiency, day 20
|
After obtaining negative gram stain, infusion of transduced PBC, day 20
|
Patient follow-up for 2 months (end of cycle 1)
|
Repeat 2 more cycles

B. G-CSF administration

To mobilize peripheral blood stem cells, patients will receive 4 daily doses of recombinant human granulocyte colony stimulating factor (G-CSF) at a dose of 16 μg/kg/day by subcutaneous injections. The first 3 doses will be administered in the

Outpatient Department before initiation of peripheral blood cell (PBC) collections. The last dose will be administered after the first collection of PBC. Injections must be scheduled to begin on a Friday, in order for leukapheresis and laboratory processing to be performed on consecutive weekdays of the following week.

C. Peripheral blood cell collection

PBC are collected by continuous flow centrifugation on days 4 (Monday) and 5 after the first dose of G-CSF with an expected yield of 5 - 10 x 10^8/kg peripheral blood mononuclear cells. Collections will take place in the FHCRC Pheresis Unit under the supervision of Dr. Bensinger. Venous access will be accomplished using a temporary percutaneous Mahurkar catheter. Patients should receive a 7-day course of dicloxacillin or another appropriate antibiotic, starting on the day of line placement.

D. Separation of CD34+ peripheral blood cells

To simplify the transduction procedure by reducing the cell number manipulated and to probably increase the efficiency of gene transduction by improving the ratio of repopulating cell to vector particles, peripheral blood mononuclear cells will be enriched for CD34+ cells using the CellPro Stem Cell Separator. Mononuclear cells will be incubated with biotinylated murine antihuman CD34 antibody 12.8, washed and passed over an avidin biogel column. The cells adhering to the column will be dislodged by mechanical agitation and collected. A sample of CD34+ cells will be saved for FACS analysis and for use as pre-transduction control for PCR and for glucocerebrosidase enzyme assay.

E. Transduction of CD34+ peripheral blood cells

CD34+ peripheral blood cells will be transduced by incubation for 5 days in a long-term marrow culture system containing vector supernatant. Adherent stromal layers of LTMC will be established with autologous marrow cells 14 days before start of transduction. LTMC will contain 50% LTMC medium: Iscove's modified Dulbecco's Medium at 365 mOsM supplemented with 12.5% fetal calf serum, 12.5% horse serum, 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, 0.4 mg/ml of L-glutamine, 0.01 mg/ml folic acid, 0.04 mg/ml myoinositol, 0.01 mM 2-mercaptoethanol, and 1 µg/ml hydrocortisone sodium succinate. LTMC will also contain rhIL-1, rhIL-3, rhIl-6, rhSCF (each at 50 ng/ml), 50% vector-containing supernatant from PG13/LgGC cell cultures and 5 µg/ml protamine sulfate. LTMC will be fed every day with 50% fresh vector-containing supernatant and 50% used LTMC medium. After 5 days, adherent and nonadherent cells will be harvested from the LTMC and washed. Cell samples will be taken to test for viability using trypan exclusion and colony assays and for sterility using gram stain, culture for bacteria and fungi, mycoplasma screen and endotoxin test. Pooled supernatants from the transduction step will be analyzed for replication competent retrovirus using an S+L- focus assay on PG-4 cells. Cell samples will also be tested for transduction efficiency by PCR, Southern blot, and glucocerebrosidase enzyme assay. Transduced cells will only be used for infusion into the patient if gram stain is negative.

F. Infusion of transduced CD34+ peripheral blood cells

CD34+ peripheral blood cells will be infused into the patient over a period of two hours or less. Patients will receive premedication 30 minutes before start of infusion with 25 mg diphenhydramine i.v., 650 mg acetaminophen p.o. and 50 mg hydrocortisone i.v. and will be closely monitored during the infusion and 4 hours after. All patients will be

monitored in the hospital overnight. If no adverse reactions are observed, they will be discharged the following day for subsequent outpatient follow-up by the referring physician.

G. Frequency of infusion of transduced CD34+ peripheral blood cells

Peripheral blood collection, transduction and infusion will be repeated two more times at two month intervals for a total of three infusions.

9. Evaluation

A. Pretransplant Evaluation

The following clinical and laboratory evaluations will be performed for all patients within 30 days before start of treatment by the referring physician (screening). These screening evaluations must be reviewed prior to initiation of treatment. Certain evaluations will be repeated on the day of infusion of transduced CD34 peripheral blood cells (Day of transplant) prior to infusion.

1. Complete history and physical examination (screening and repeat physical exam on Day of transplant).
2. Karnofsky Performance Status (screening).
3. Chest x-ray (PA and LAT) (screening).
4. Electrocardiogram (screening).
5. Urinalysis (screening and repeat on Day of transplant).
6. Serum Chemistries: uric acid, calcium, phosphorus, magnesium, amylase, transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, triglycerides, BUN, creatinine, albumin, total protein, electrolytes, glucose, non-tartrate inhibitable acid phosphatase and angiotensin converting enzyme (screening and repeat Day of transplant).
7. Hematology: Complete blood count (CBC), differential, platelets, PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen (screening, during G-CSF treatment, and on Day of transplant).
8. Pregnancy Test: Serum pregnancy test must be negative within 7 days prior to Day of transplant.
9. HIV Negative Antibody Test: ELISA (screening).
10. HBsAG: Hepatitis B surface antigen determination (Screening).
11. Liver-Spleen Size by MRI or CAT: In patients with clinical hepatosplenomegaly.
12. Resting transcutaneous oxygen saturation: Must be > 90% on room air.
13. Glucocerebrosidase enzyme level: collect PBMCs for this determination (screening).
14. Freezing Specimens: Serum, plasma, and blood cells will be separated and frozen as baseline (Day of transplant).
15. DNA/RNA Analysis: Circulating mononuclear cells will be analyzed using PCR-related techniques for the presence of vector gene sequences (Day of transplant).
16. Marrow MRI Analysis: In patients with bone involvement.
17. X-ray of bones affected by disease.
18. Glucocerebrosidase gene mutation.

(Send samples for 13-15 and 18 to Dr. Schuening's lab.)

B. Posttransplant Evaluation

The following clinical and laboratory evaluations will be performed for all patients after infusions of transduced peripheral blood cells at monthly intervals during the first year after transplantation, every 3 months during the second year after the last infusion and then yearly unless there is clinical reason to see the patient more often.

1. Physical Examination.
2. Karnofsky Performance Status.
3. Urinalysis.
4. Serum Chemistries: Uric acid, calcium, phosphorus, magnesium, amylase transaminases (SGOT, SGPT), alkaline phosphatase, LDH, total bilirubin, triglycerides, BUN, creatinine, albumin, total protein, electrolytes, glucose, non-tartrate inhibitable acid phosphatase and angiotensin converting enzyme.
5. Hematology: Complete blood count (CBC), differential, platelets, PT (Prothrombin Time), PTT (Partial Thromboplastin Time), and fibrinogen.
6. Pregnancy Test: Serum pregnancy test will be repeated monthly during the first year.
7. Liver-Spleen Size: Optional, based on clinical assessment.
8. Glucocerebrosidase enzyme level determination: Collect PBMCs.
9. S+L-assay for helper virus.
10. Freezing Specimens: Additional serum, plasma, and cells will be separated and frozen.
11. DNA/RNA Analysis: Circulating mononuclear cells will be analyzed using PCR-related Techniques for the presence of vector gene sequences.
12. Glucocerebrosidase gene mutation.
13. Marrow MRI analysis: Analysis of marrow involvement will be performed if clinically indicated.
14. X-ray of bone affected by disease.
    (Send samples for 8-12 to Dr. Schuening's lab.)

C. Evaluation upon premature withdrawal

1. Discontinued patients who have not received transduced cells will not be followed after they are discharged.
2. Patients who are discontinued or withdraw before completing all cycles, but have completed at least 1 cycle (patient was reinfused with transduced cells), will be asked to return to the clinic to be evaluated according to section B.

D. Autopsy Request:

It is very unlikely that a patient will die during their participation in this protocol. Should death occur, an autopsy will be requested to determine the extent of gene transfer into important organs such as marrow, liver, and spleen.

10. Special Considerations

A. Adverse Reaction Reporting Requirements

This protocol uses retrovirus-mediated genetic material which is considered an IND agent. Toxicities that are not transplant related should be considered IND related. The following adverse reactions must be reported by the principal investigator or his associates to the NIH Office for Protection from Research Risks (OPRR) and the local

IRB in the following manner described. In addition, a copy of this report will be forwarded to the NIH Office of Recombinant DNA Activities and the US Food and Drug Administration.

Deaths. Any death from any cause which is not transplant related and that might possibly be related to gene transduction, must be reported as follows:

The principal investigator or one of his associates must call the OPRR, Charles R. McCarthy, (301) 496-7005, within 24 hours of the event. An adverse Drug Reaction Form must be sent to the OPRR within 10 working days.

Charles R. McCarthy
OPRR
Building 31, Room 5B-59
NIH
Bethesda, MD 20892

Life-threatening events (grade 4 toxicity)
The principal investigator or one of his associates must call the OPRR, Charles R. McCarthy, (301) 496-7005, within 24 hours of any grade 4 toxic event which may be due to the IND agent. An adverse Drug Reaction Form must be sent to the OPRR within 10 working days.

First occurrence of a toxicity. The first occurrence of a toxicity (not previously reported, no matter what the grade) felt to be related to gene transduction, must be reported by the principal investigator or one of his associates by phone to the OPRR, Charles R. McCarthy (301) 496-7005, within 24 hours of the event. An Adverse Drug Reaction Form must be sent to the OPRR, Charles R. McCarthy, within 10 working days.

B. Informed Consent
The principal investigator or one of his associates must explain verbally and in writing the nature of the study and the procedures of GC gene transfer into PBRC in such a manner that each patient is aware of the potential risks. The patient must also be informed that he/she may withdraw from the study at any time and for whatever reason, without prejudice to their future treatment.

11. Records

Clinical Statistics maintains a patient database at FHCRC to allow storage and retrieval of patient data collected from a wide variety of sources. The investigator will ensure that data collected conform to all established guidelines for coding, collection, keyentry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Any publication or presentation will refer to patients by this number and not by name. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are kept in a locked room. They are maintained by the FHCRC data collection staff which is supervised by an A.R.T. Access is restricted to personnel authorized by the Division of Clinical Research. All required clinical evaluation records, analysis of the

clinical outcome and toxicity, and the laboratory evaluation of retrovirus insertion will be the responsibility of Dr. Friedrich Schuening.

12. Statistical Considerations

The primary endpoint of this study is to examine the safety of ex vivo retrovirus-mediated transfer of the human GC cDNA into peripheral blood repopulating cells of patients with type 1 Gaucher's disease followed by infusion of the transduced cells into the patients. The persistence and expression of the GC gene in hematopoietic cells after infusion will also be studied. At the conclusion of the study, each of these parameters will be presented descriptively. A total of 10 patients at least 18 years of age having documented type 1 Gaucher's disease will be enrolled onto the study. With respect to safety, we do not expect any adverse events like helper virus generation or retrovirus induced tumor development to occur. If 10 patients are treated and none experiences adverse events, then the upper limit of the 80% one-sided confidence interval for the true probability of an adverse event is 14.8%, i.e., if no adverse events occur among the 10 patients, we can be 80% confident that the true risk of an adverse event is less than 14.8%.

13. Termination of the study

No toxicities related to gene transduction were observed in patients who were treated with infusions of tumor-infiltrating lymphocytes which had been marked with a *neo* gene containing retrovirus vector (LNL6) very similar to the vector LgGC used in this study. The study will be terminated if at any time the probability of any grade 3 toxicity that is felt to be due to the gene therapy procedure, including the development of replication-competent murine retrovirus in patients, exceeds 10% as determined by the lower limit of an 80% one-sided confidence interval that exceeds 10%. This will occur if 1/2 or 2/4-8 patients experience the adverse event. If any patient experiences a fatal toxicity due to the gene therapy, or develops a tumor which contains retrovirus-vector DNA or replication-competent virus DNA, then the study will be terminated, as any non-zero incidence of gene therapy related grade 4 toxicity is unacceptable.

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#838.0

FRED HUTCHINSON CANCER RESEARCH CENTER
AND THE UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE, DIVISION OF ONCOLOGY

Consent to Participate in a study on Retrovirus-Mediated Transfer of the cDNA for Human Glucocerebrosidase into Peripheral Blood Repopulating Cells of Patients with Gaucher's Disease.

Principal Investigator: Friedrich Schuening, M.D., Assistant Member, FHCRC (667-4410).
Associates: Jean Sanders, M.D., Professor of Pediatrics, UW, Member, FHCRC (667-4348); C. Ronald Scott, M.D., Professor of Pediatrics, UW (548-3370); Rainer Storb, M.D., Professor of Medicine, UW, Member, FHCRC (667-4407); A. Dusty Miller, Ph.D., Affiliate Associate Professor of Pathology, UW, Member, FHCRC (667-2890); John A. Barranger, M.D., Ph.D., Professor of Pediatrics, University of Pittsburgh; William Bensinger, M.D., Associate Professor of Medicine, UW, Associate Member, FHCRC; Scott Rowley, M.D., Associate Member, FHCRC; Ted Gooley, Ph.D., Assistant Member FHCRC; Hans-Peter Kiem, M.D., Fellow, FHCRC; Christof von Kalle, M.D., Visiting Scientist, FHCRC; Fred Appelbaum, M.D., Professor of Medicine, Member, FHCRC.

Phone: Working Hours (206) 667-4410; Emergency (24 hour) Phone: 667-5001

Attending physician: _____ Phone: _____

BACKGROUND

Gaucher's disease is caused by a missing gene in your body. A gene is the part of a chromosome (hereditary material) that contains the information needed by cells to make proteins. The gene which is missing in your body is responsible for producing an enzyme called glucocerebrosidase or GC for short. The purpose of the GC enzyme is to break down a lipid or "fat" in the cells of your body. If GC is missing, this lipid accumulates in the cells of the body, mainly in the cells of the spleen, liver and bone marrow. The accumulation of the lipid in the spleen and the liver often causes these organs to become very large. A large spleen can cause bleeding because more platelets, which are necessary to prevent bleeding, are being destroyed in the enlarged spleen. Enlargement of the liver can reduce the normal function of the liver. A large liver and spleen can make breathing more difficult. The accumulation of lipid in the bone marrow can cause bone pain, deformities and fractures of bones.

Currently two forms of treatment are available for patients with Gaucher's disease. One form is bone marrow transplantation. Marrow cells are obtained from a brother or sister that does not have Gaucher's disease and that has the same type of tissue markers as the patient. Transplantation of marrow obtained from such a "matched" sibling is often successful in improving the problems of Gaucher's disease. However, only about 1 of every 4 patients will have such a "matched" sibling donor. In addition there is about a 10-20% chance of dying from complications of the marrow transplant procedure.

The second form of treatment currently available for patients with Gaucher's disease is to give the missing enzyme GC to the patient in form of the medication "Ceredase". This treatment is safe and effective, but it needs to be continued for the entire life of a patient. Most of all, this medication is very expensive, costing about $100,000-$400,000 per patient per year depending on the patients body weight. It is therefore necessary to look for new forms of treatment of Gaucher's disease which are safe and affordable.

PURPOSE

The purpose of this study is to investigate a new form of treatment of Gaucher's disease called "gene therapy". We will obtain blood cells from you which contain a small number of cells called "stem cells". These stem cells make all the different blood cells such as red blood cells, white blood cells and platelets. White blood cells are the cells of the blood which are most effective in fighting infections. Platelets are important to prevent potentially life-threatening bleeding. One type of the white cells made by the stem cells are called "macrophages". The macrophages of patients with Gaucher's disease are the cells which store up the body lipid (a kind of fat) because they lack the GC enzyme. We will insert the normal gene for the missing GC enzyme into your stem cells. If we are successful, the stem cells which contain the GC gene will then produce blood cells which also contain the GC gene including macrophages. We expect that these new macrophages which contain the gene for the GC enzyme will then no longer accumulate lipid but that the GC enzyme now present in these cells will break down this fatty material. This should lead to improvement of problems typical for Gaucher's disease like enlargement of liver and spleen as well as bone problems. There is no guarantee that this procedure will be successful.

PROCEDURES

Twenty days before infusion of your genetically altered blood cells, approximately 20 ml of marrow blood (4 teaspoons) will be drawn. These cells will be used to establish a layer of cells in culture flasks which will later feed your blood stem cells while the GC gene is inserted in them. Ten days after marrow aspiration, you will receive a catheter (small plastic tube) inserted in one of your large veins which makes it easier to collect your blood cells later. On the same day, you will also start receiving treatment with a growth factor (a substance which stimulates the growth of marrow and peripheral blood mother cells which can make red cells, white cells and platelets) called G-CSF. This factor will be given daily for four consecutive days. It will be injected underneath your skin. After four days, your peripheral blood cells contain an increased number of mother cells or stem cells. At this time, your blood cells will be collected on two consecutive days by connecting your catheter to a machine which separates white blood cells (leukopheresis). The stem cells are then purified from the white cells in the laboratory. Subsequently your blood cells will be treated in the laboratory for 5 days with a virus, which is engineered to carry the GC gene into your blood stem cells. Once the GC gene is in your stem cells, they will pass the GC gene on to all their daughter cells including the macrophages. The macrophages will then be able to break down the fatty material called lipid because they are now able to make the GC enzyme. The virus which carries the GC gene into your stem cells has been altered such that it cannot grow or spread in your body.

After having inserted the GC gene into your blood cells, we will give your treated cells back into your body by infusing them into your vein through your catheter. The infusion will take place in the hospital where you will stay for one night, and you will be monitored closely for side effects during and after the infusion. Following the infusion you will be seen as an outpatient every month. We will obtain about 30 ml of blood (6 teaspoons) and test your blood cells whether they contain the GC gene and whether the gene is producing GC enzyme. We will repeat the whole procedure of collecting your blood cells and transferring the GC gene into them two more times.

Since this gene therapy procedure represents a new treatment for patients with Gaucher's disease, it is important for us to observe you for at least five years so that we can evaluate the further course of your

disease and monitor for any possible long-term side effects of the gene therapy procedure. During the first year we would like to see you at monthly intervals after transplantation and during the second year every three months and then yearly unless there is a specific reason to see you more often. In the very unlikely event of your death we would like to request an autopsy for the purpose of determining whether the GC gene has been transferred into important organs such as marrow, liver and spleen. Your participation in this study will in no way alter the standard care you may be receiving. If you are currently receiving enzyme replacement therapy it will continue. Depending on how much of the GC enzyme is produced by your transplanted cells we may consider trying to reduce the amount of enzyme replacement therapy you are receiving. There will be no charge to you or your insurance carrier for any of the tests, procedures or hospital stays associated with this study. You will continue to be billed for the costs of standard care including enzyme replacement treatment.

RISKS, STRESS, OR DISCOMFORT

There are some potential risks to this procedure. Even though the virus used to insert the GC gene into your peripheral blood cells cannot grow and is considered harmless to you, it is possible that events could occur within the cell that would allow the virus to grow. To minimize this possibility, the preparations of virus used to insert the GC gene into your peripheral blood cells will be tested for the presence of infectious virus before being used to genetically alter your blood cells. However, since this is a new procedure we do not know whether or not the marked cells could become abnormal after they have been injected. There is a very remote possibility that the virus that is placed into your blood cells could cause the development of cancer in your body. In our opinion this risk is extremely low. You will therefore be monitored for the presence of infectious virus which may be associated with the development of cancer. In a number of animal experiments we have been unable to demonstrate either infectious virus production or the development of cancer due to infectious virus during an observation time of up to four years after treatment. In addition, in over 50 patients who have so far received cells treated with this type of virus, no safety hazards were recognized.

In preparation for collecting your white cells as described above you will be treated with a special medication which stimulates the bone marrow to release bone marrow making cells into your blood. This medication is called G-CSF. It can cause temporary bone pain which would then be treated with pain medication. No major side effects of G-CSF have been seen so far. The collection of your white cells requires you to have a plastic tube placed in one of your veins. This tube is inserted into your chest and is necessary to handle the large volume of blood from which the white blood cells will be separated. There is a small chance of bleeding or accidentally entering the chest cavity. If this happens a tube will need to be placed in your chest for several days. This risk is very small as your catheter will be placed by an experienced physician. Collecting white blood cells (leukopheresis) requires that you are connected to a machine which separates and collects your white cells while at the same time returning your red blood cells to you. This procedure is without any major side effects. One potential minor side effect is that your red and white blood cell counts could be slightly lower at the end of the procedure. Therefore your blood counts will be monitored each morning to minimize this event to happen. The leukapheresis procedure will be repeated on four consecutive days.

At the time the blood cells are infused back into your blood stream you may experience fever, chills, difficulty breathing, and rarely a severe allergic reaction that can lead to death. You will be treated for such an allergic reaction if it should occur.

We emphasize that this procedure, called retrovirus mediated gene transfer, has only been used before in about 50 patients. Because this procedure is new, it is possible that despite our extensive efforts other unforeseen problems may occur including the very remote possibility that death may occur.

BENEFITS

At the current point in time, we do not know whether you will have any benefit from participating in this study. If we are successful in inserting the GC gene in a sufficient number of your blood stem cells such that part of your blood cells start producing GC enzyme, this could lead to improvement of your disease. If you are receiving enzyme treatment and we can find cells in your blood which produce GC enzyme, our plan would be to slowly withdraw enzyme treatment over a period of 9 months to see whether withdrawal of enzyme replacement can be tolerated. Currently there is no certainty that the gene therapy approach being used in this study will be successful. It is possible, however, that results of this study will lead to a new therapy which may benefit you in the future.

ALTERNATIVES

The alternatives are not to participate in this research study.

OTHER INFORMATION

Your decision to participate in this study is voluntary. You can decide whether or not to participate in this study. You may decide not to participate in this study at any time, for any reason, without notice. You will continue to receive medical care even if you decide to discontinue treatment on this study. You will not be charged nor will you be paid for your participation in this study, although you will be charged for the medical and hospital care you require independent of this study just as though you were not part of this study.

In order to evaluate the results of this study, your medical records will need to be available, now and in the future, to other physicians and researchers associated with the research project. The National Institutes of Health, the Food and Drug Administration will also have access to this information. All precautions to maintain confidentiality of medical records and personal information will be taken. Your personal identity will not be revealed in any publication or results. Study records will be maintained indefinitely for the purpose of analysis and follow-up.

If you have questions about the research or a research related injury, please contact Friedrich Schuening, M.D. (667-4410) or your attending physician.

If you have any questions about your rights as a research participant, please contact Karen Hansen in the Institutional Review Office of Fred Hutchinson Cancer Research Center at 206/667-4867.

Investigator's Statement

I have provided an explanation of the above research program. The subject was given an opportunity to discuss the procedures, including possible alternatives, and to ask any additional questions. A signed copy of this consent form has been given to the subject.

_____

Investigator's Signature /Date

Subject's Statement

I agree to this study. I have had an opportunity to ask questions of the physicians, including questions about risks, benefits and alternatives to treatment. I have also had the opportunity to ask questions about the study, my participation and the need for access to my medical records. They have been answered to my satisfaction. I understand future questions I may have about the research will be answered by one of the investigators listed above and that any questions I have about my rights as a research subject will be answered by the person identified above. No promises or guarantees have been made regarding the anticipated outcome of any tests or procedures. I am aware that I will not be charged nor will I be paid for my participation in this study although I will be charged for the medical and hospital care I require independent of this study just as though I were not part of this study.

I give permission for my medical records to be available to physicians and personnel for this study at the University of Washington and the Fred Hutchinson Cancer Research Center, the National Institutes of Health, and the Food and Drug Administration. I acknowledge that I will receive a signed copy of this consent form.

Patient	/Date	Witness	/Date
Parent/legal guardian [for patients < 18 years old]	/Date	Other parent/legal guardian [if reasonably available]	/Date

#838.0 - 09/28/93

Copies to: Patient, Medical Records, Research file

Signed Consent MUST be sent to Data Management - Eklind 206

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Friedrich SCHUENING	Assistant Member, FHCRC

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
University of Mainz, Germany	BA	1968	Theology
University of Hamburg, Germany	MD	1976	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1976-1976 Intern, University Hospital of Hamburg, West Germany
1977-1979 Resident in Internal Medicine, Allgemeines Krankenhaus, Hagen, West Germany
1979-1983 Staff Specialist in Medical Oncology, West German Cancer Center, Essen, West Germany
1984-1986 Visiting Scientist, Fred Hutchinson Cancer Research Center, Seattle WA
1986-1990 Associate in Clinical Research, FHCRC, Seattle WA
1990-date Assistant Member, Clinical Research Division, FHCRC, Seattle, WA

Publications (selected from over 80)

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HUMAN GENE TRANSFER PROTOCOL

9312-062 Haubrich, Richard; University of California at San Diego Treatment Center, San Diego, California; *An Open Label, Phase I/II Clinical Trial to Evaluate the Safety and Biological Activity of HIV-IT(V) (HIV-1 IIBenv/Retroviral Vector) in HIV-1 Infected Subjects.*

Date of RAC Approval: 12/3/1993
Date of NIH Approval: 4/19/1994

SCIENTIFIC ABSTRACT OF THE STUDY

This gene therapy protocol involves the use of retroviral vector-mediated gene transfer as treatment for human immunodeficiency virus (HIV)-infected individuals. The protocol employs a genetically engineered, non-replicating, amphotropic murine retroviral vector (N2 IIIBenv) encoding the HIV-1 IIIB envelope (ENV and REV) proteins. Preclinical studies have demonstrated the ability of the N2 IIIBenv/rev vector to induce immune responses in mice, Rhesus monkeys, and baboons. Specifically, the N2 IIIBenv/rev vector was capable of inducing HIV-1 IIIBenv/rev-specific $CD8^+$ cytotoxic T lymphocyte (CTL) and antibody responses in these animals. Murine CTL induced by vector transduced cells also exhibited crossreactivity by lysing cells infected with different HIV-1 prototypic strains and clinical isolates. A balanced *in vivo* immune attack by HIV-specific CTL and antibody responses would be expected to eliminate HIV-infected cells and clear cell-free virus, respectively, from an infected individual.

The Phase I/II clinical trial involves the direct administration of the N2 IIIBenv/rev retroviral vector to HIV-infected, seropositive, asymptomatic individuals. The direct vector treatment consists of a series of three monthly intramuscular injections of the test article. The packaging cell line, producer cell line, and processed vector material have undergone extensive quality control analysis for the presence of contaminating agents. Treated individuals will be evaluated for acute toxicity and for routine clinical parameters, CD4 levels, HIV-specific CTL responses, and viral load prior to, during, and following treatment. The treated subjects will also be requested to participate in follow-up for at least three years to identify long-term treatment effects and to evaluate their disease progression.

The study protocol is designed to initially evaluate the safety of the direct administration of retroviral vector-mediated gene therapy as a treatment for individuals with a life-threatening disease. However, functional, biological and clinical disease parameters will be monitored as well to provide a basis for additional human studies.

NON-TECHNICAL ABSTRACT OF THE STUDY

Human Immunodeficiency Virus (HIV) is a virus which has the ability to invade and damage cells of the immune system. It infects and kills that specific group of white blood cells called $CD4^+$ T cells. The result of this loss is a serious disabling of the body's immune system, leaving the infected individual open to infections and cancers.

The body also has specific white blood cells known as cytotoxic T lymphocytes (CTL), that function as important killers of cells infected with viruses. HIV-infected individuals who are in an early stage of disease and who are symptom-free could theoretically benefit from a treatment that stimulates these CTL.

Scientists have recently developed a way to insert new genetic information into human cells through use of a disabled virus, called a retroviral vector. The retroviral vector has been designed such that it cannot reproduce to cause disease, but delivers to target cells the genetic codes for the production of certain proteins that resemble important proteins of HIV. The retroviral vector instructs the human target cells to produce such therapeutic proteins. These proteins can then lead to enhanced immune responses targeted at killing HIV-infected cells.

Experiments in mice and non-human primates have shown that treatment with these specific retroviral vectors can stimulate the production of HIV-specific antibodies and CTL without producing any toxic side effects.

This study is designed to determine the safety of the retroviral vector encoding HIV genes for use in humans, and, of secondary importance, whether the vector will have the ability to trigger CTL responses in humans as it does in laboratory animals.

Title: **An Open Label, Phase I/II Clinical Trial to Evaluate the Safety and Biological Activity of HIV-IT (V) (HIV-1 IIIB*env/rev*-Retroviral Vector) in HIV-1-Infected Subjects**

Protocol

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VIAGENE HIV-I-03-393

PROTOCOL VERSION 1.4

Title: **An Open Label, Phase I/II Clinical Trial to Evaluate the Safety and Biological Activity of HIV-IT (V) (HIV- 1 IIIB *env/rev*-Retroviral Vector) in HIV-1-Infected Subjects**

1.0 Objectives

1.1 Three monthly intramuscular injections of HIV-1 Immunotherapeutic vector [HIV-IT (V)] at a dose of approximately 10 million colony forming units (10^7 cfu) will be administered to determine:

1.1.1 Safety and tolerability of three monthly injections of HIV-IT (V).

1.1.2 The effects on the specific cytotoxic T lymphocyte (CTL) response to HIV-1.

1.1.3 Effects on HIV-1 antibody levels.

1.1.4 Effects on viral load.

1.1.5 Effects on $CD4^+$ and $CD8^+$ T-cell subpopulations.

1.1.6 Early clinical indications of effects on disease progress.

2.0 Background and Rationale

2.1 Immune Response to HIV-1

2.1.1 Both cellular and humoral immune responses have been documented in HIV-1 infected individuals. During acute HIV-1 infection, high titers of virus are present in the blood as established by cell culture and p24 detection. Recent studies have shown that CTL responses directed against GAG, POL, and ENV antigens occur earlier than neutralizing antibody (NA) responses and appear to mediate the decrease in viral load.[1]

2.1.2 The protective effect of anti-HIV-1 NA is uncertain. Despite the presence of NA directed against the V3 loop and $CD4^+$ combining site of gp120, clearance of HIV-1 infection does not occur. HIV-1 can evade NA by direct cell to cell spread.[2] The V3 loop shows considerable inter-strain variability and antibodies directed against this region are type specific but not group specific. Viral mutation in this region can lead to escape from the NA response. In addition, there has been no correlation between the level of NA and HIV-1 disease progression.

2.1.3 To date, most immunotherapeutic vaccines have been targeted at the development of new or increased levels of antibody. There is now increasing evidence of the importance of CTL in preventing HIV-1 spread. The ability to increase CTL activity may be a potential method of controlling progression of HIV-1 infection.[3]

2.1.4 HIV-1 is a virus which can confound the immune system as follows: (1) Gene segments coding for the envelope protein mutate frequently, including those portions of the envelope which are immunodominant with respect to antibody production[4], (2) HIV-1 may down-regulate expression of major histocompatibility complex (MHC) molecules

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[1]Safrit, et al., *J. Cell Biochem.* Suppl. 17E:Q146 (1993).

[2]Popovic, M., et al., *Science*, 224:497-500 (1984).

[3]Walker, B.D., et al., *AIDS*, 4:177-184 (1990).

[4]La Rosa, G. J., et al., *Science*, 249:932-935 (1990).

and antigen presentation *in vitro* in cells in which HIV-1 virions are replicating.[5] If MHC down regulation occurs *in vivo,* this may be responsible for lower levels of CTL induction, because MHC molecules present foreign antigens to the immune system, and (3) HIV-1 infects and disables CD4 T-cells, which results in the progressive functional loss of this critical immune cell population as the infection spreads.[6] The virus can heavily infect CD4 cells in lymph nodes even in the early stages of infection.[7] The loss of CD4 cells correlates with profound immunosuppression, leaving the subject unprotected against opportunistic infections and cancers, which lead to the morbidity and mortality of HIV-1 infection.

2.1.5 Given these tactics by the virus, it is not altogether surprising that the therapeutic contributions made by humoral and cellular immunity in controlling the pathologic effects of HIV-1 infection are poorly understood. HIV-1 specific antibodies, some of which possess neutralizing activity *in vitro* [8], are generated during the natural course of HIV-1 infection. NA may eliminate extracellular virus *in vivo*, but HIV-1 can also spread by cell-to-cell fusion. Thus HIV-1 infection and spread may continue in the presence of antibodies.

2.1.6 T-cell-mediated immunity, involving the induction of $CD8^+$ CTL that directly destroy virus-infected cells, has been shown to be important in limiting the severity and duration of several viral diseases[9]. $CD8^+$ CTL, capable of lysing target cells expressing HIV-1 envelope, gag, polymerase and nef proteins *in vitro* have been detected in HIV-1 seropositive subjects, and may be important in controlling HIV-1 infection *in vivo*[10]. Although the precise impact of the immune response on HIV-1 infection remains to be elucidated, it is likely that the HIV-1 viral infection and replication process does not permit the full therapeutic benefit of the immune response to be invoked.

2.2 Rationale for an HIV-1 Gene Transfer Immunotherapeutic

2.2.1 The rationale for use of HIV-IT (V) is to stimulate the production and activity of HIV-1 specific $CD8^+$ CTL above levels induced by natural infection. Some of the CTL may be stimulated against novel epitopes and provide cross-strain protection from progressive HIV-1 infection. Previous immunotherapeutic studies have relied on soluble antigen (i.e. gp160) or inactivated virus as immunogens. Theoretically, these antigens would boost humoral immune responses.

2.2.2 Generally, a long, symptomless period follows the initial acute infection with HIV-1.[11] Infected individuals could, therefore, benefit from an immunotherapy that stimulates augmented HIV-1 specific $CD8^+$ CTL. $CD8^+$ CTL are efficient "killers" of virally infected cells.[12] Class I MHC restricted $CD8^+$ CTL represent the arm of the immune system that has been specifically developed to combat viral infections.[10] HIV-IT (V) is a promising agent for clinical trials as a treatment in HIV-1 infection because injecting HIV-IT (V) has been shown to specifically induce the CTL immune responses of mice and of non-human primates. HIV-IT (V) also elicits an antibody response in both murine and primate systems directed against the HIV-1 IIIB envelope protein.

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[5] Scheppler, J.A., et al., *J. Clin. Invest.*, 72:398 (1983).

[6] Rosenberg, Z.F., et al., *AIDS Res. and Human Retroviruses*, 5:104 (1989).

[7] Pantaleo, G., et al., *Nature* 362:355-358 (1993).

[8] Robert-Guroff, M., et al., *Nature*, 316:72-74 (1985)

[9] Rouse, R.T., *Rev. Infect. Dis.*, 10:16-33 (1988).

[10] Koenig, S., et al., *J. Immunol.* 145:127-135 (1990).

[11] Robert-Guroff, M., et al., *Nature*, 316:72-74 (1985).

[12] Klavinskis, L.S., et al., *J. Virol.*, 63:4311-4316 (1988).

2.3 Description of HIV-IT (V)

2.3.1 HIV-IT (V) is an HIV-1 immunotherapeutic based on retroviral vector-mediated gene transfer technology that provides for the delivery of the *env* and *rev* gene segments of the HIV-1 IIIB strain directly into subject cells. The retroviral vector containing these genes and the vector-producing cell line are subjected to Quality Control testing prior to injection into multiple intramuscular (IM) sites. Proteins expressed from these genes and presented on the surface of transduced recipient cells may cause substantial stimulation of immune responses, in particular the $CD8^+$ CTL response.

2.3.2 The safety issues regarding retroviral vector gene therapy have been addressed in terms of final product testing as well as the molecular design of the vector producer cell line. The cell line which produces HIV-IT (V) (DA/N2 IIIBenv.2C3) has been characterized and subjected to numerous quality control analyses, including assays to detect replication-competent retroviruses (RCR).

To increase safety, the DA/N2 IIIBenv.2C3 producer cell line (PCL) is derived from a canine cell line with a background genome that contains no detectable sequences homologous with the vector or the vector particle structural genes.

The PCL contains genetically unlinked murine leukemia virus (MLV) structural genes (split genome) to reduce the possibility of homologous recombination between the provector DNA and the DNA encoding the murine retrovirus structural genes (gag/pol and env_{am}) present in the PCL.[13]

In addition, there is reduced sequence homology between the MLV structural genes and provector (N2 IIIBenv) DNA because the structural genes in the producer cell line use an heterologous transcriptional promoter and termination signals instead of Moloney murine leukemia virus long terminal repeats (MoMLV LTRs). Multiple homologous recombination events within minimal sequence overlap would be required to generate a RCR.

Several procedures have been used at Viagene to detect RCR in conjunction with a commercially available validated extended S^+L^- test. Vector preparations are tested for release using the extended S^+L^- assay. A sample of post production PCL is tested for RCR using a *Mus dunni* cocultivation procedure. Cocultivation testing on the producer cell line before banking, and on the banked cell line have also been carried out. All samples tested have yielded uniformly negative results for RCR. When all tests of liquid HIV-IT (V) are negative for RCR, HIV-IT (V) undergoes final formulation and lyophilization. The lyophilized final product must also pass a battery of quality control tests.

2.4 Preclinical Research and Toxicology/Pharmacology Studies

2.4.1 Viagene and the scientific staff at the FDA's Center for Biologics (CBER) have worked jointly to develop a plan to evaluate the safety and biologic activity of HIV-IT (V). Studies have been carried out in mice and non-human primates to address issues of safety, toxicity, vector localization, and biologic activity of HIV-IT (V).

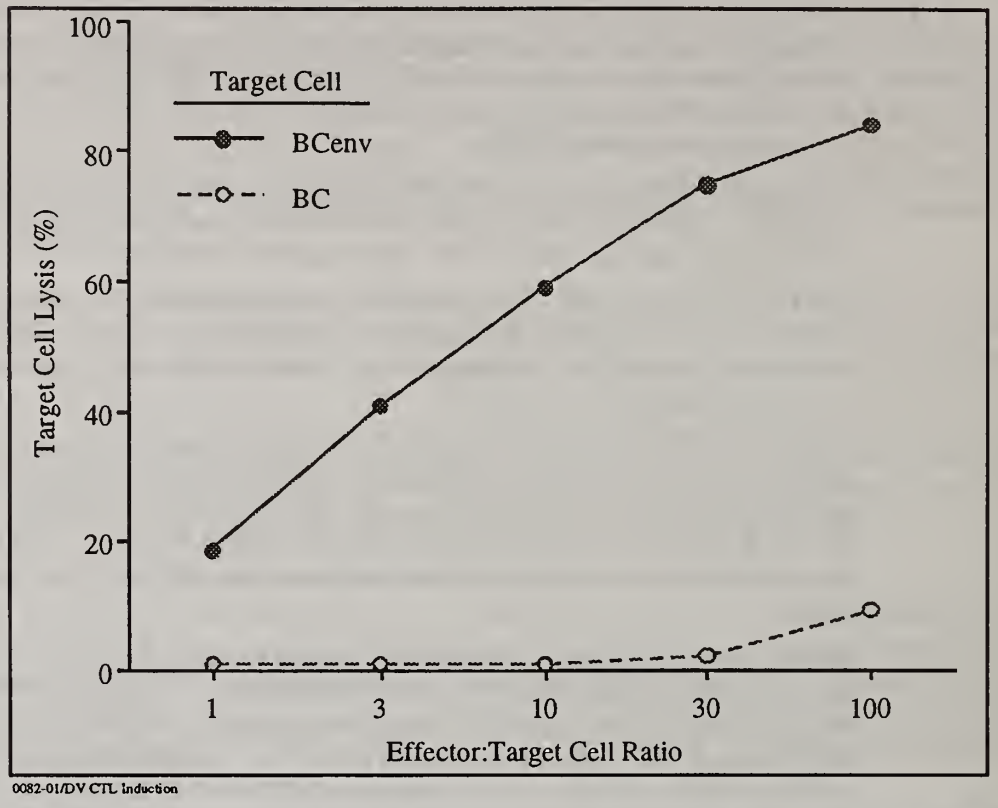
2.4.2 Initial preclinical studies were conducted in a mouse model. The mouse system has been used to evaluate the induction of humoral and cellular immune responses following direct administration of HIV-IT (V). Briefly, mice were immunized with a series of HIV-IT (V) injections and HIV-1 ENV/REV specific $CD8^+$ CTL responses were analyzed.

Representative data from responding mice are shown in Figure 1 where HIV-IT (V) injected into BALB/c mice induced a CTL response directed against target cells

[13]Miller, Human Gene Therapy, 1:5-14 (1990).

expressing the HIV-1 IIIB ENV/REV proteins (BCenv), whereas no significant lysis of the control BC cells was observed. Results from additional studies have indicated that: (1) multiple (two or more) injections are preferable, (2) the intramuscular (IM) route is adequate, (3) the cytotoxic effector cell population is comprised of $CD8^+$, Class I MHC restricted CTL, and, (4) the specificity of the CTL response is directed against the HIV-1 ENV and REV protein determinants.

Figure 1
HIV-IT (V) CTL Induction

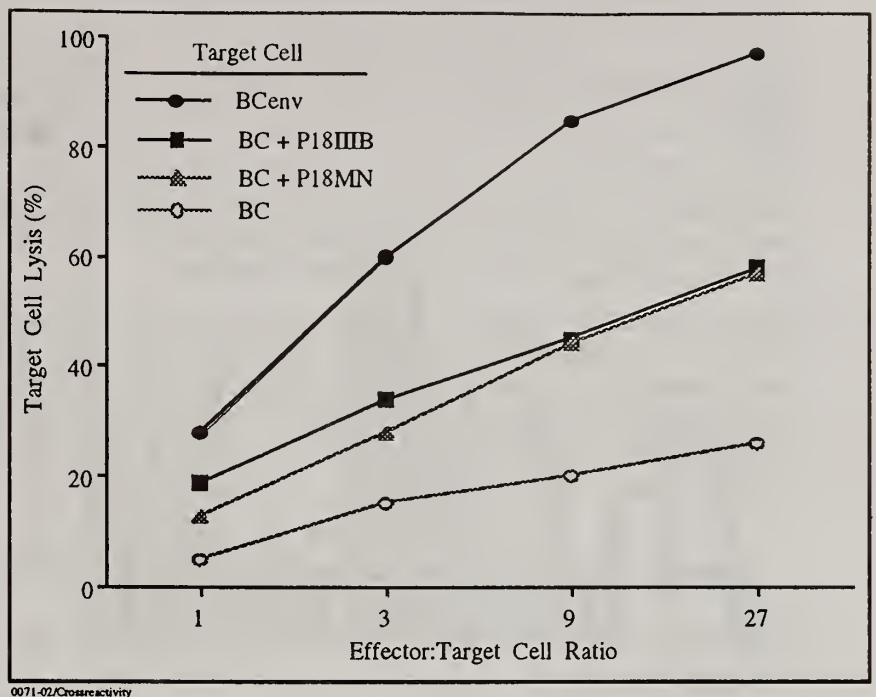


CTL Induction in BALB/c mice injected IM with HIV-IT (V). Stimulated effector cells were tested on HIV env/rev-expressing (BCenv) and control (BC) target cells using a standard ^{51}Cr-release cytotoxicity assay.

CTL induced with HIV-IT (V) show cross-reactivity against determinants of different HIV-1 isolates. HIV-IT (V)-induced CTL are capable of lysing target cells coated with a peptide (P18 IIIB) derived from the V3 region of the HIV-1 IIIB envelope protein and exhibit cross-reactive lysis of target cells coated with the P18MN peptide derived from the equivalent V3 region of the HIV-1MN envelope protein (Figure 2). Similar CTL cross-reactivity directed against human/mouse-MHC target cells infected with different, more prevalent U.S. prototypic viral strains and clinical HIV-1 isolates has been demonstrated following immunization of mice with syngeneic cells transduced with HIV-IT (V). In contrast to type-specific antibody responses, the HIV-IT (V)-induced CTL response appears to exhibit broad reactivity against cells infected with diverse HIV-1 strains.

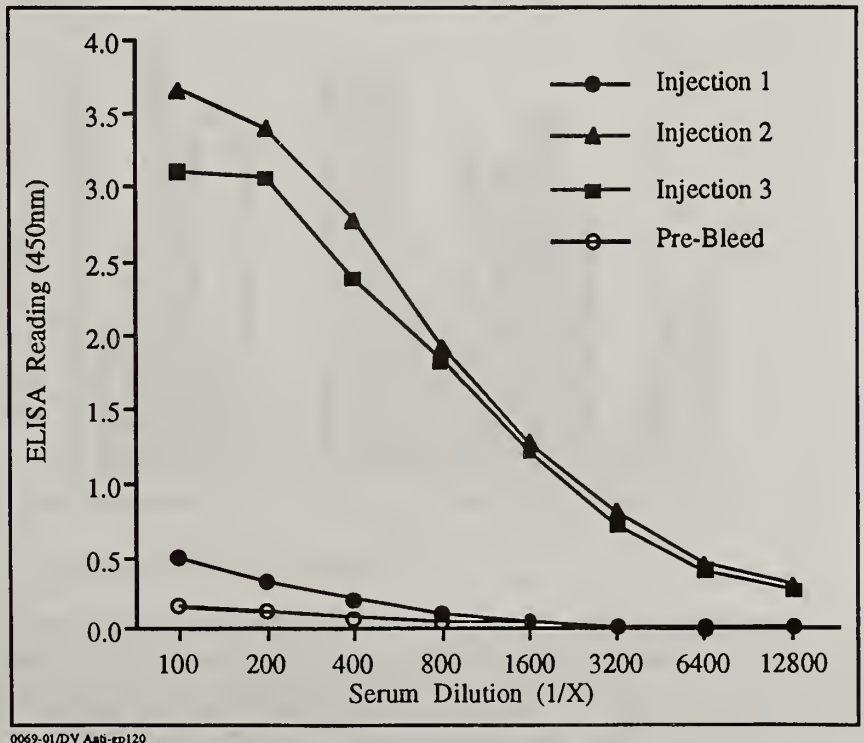
Mice treated with HIV-IT (V) have also produced antibodies that specifically react to the HIV-1 IIIB envelope protein. Mice were immunized with up to three intramuscular injections of HIV-IT (V) and their serum tested for reactivity (binding) to gp120 (Figure 3). Other studies have shown that antibodies that bind to a peptide derived from the V3 region of the HIV-1 IIIB envelope protein do not cross-react with a peptide derived from the equivalent region of the HIV-1 MN isolate.

Figure 2
Crossreactivity of HIV-IT (V)-Induced CTL



HIV-IT (V)-induced CTL recognize HIV-1 IIIB and HIV-1 MN V3 region determinants. Data reported above were generated from a CTL line established through *in vitro* stimulations with the BCenv cell line and Hu-IL2. CTL were tested on BC targets coated with peptides derived from the V3 envelope region of the HIV-1 IIIB (P18 IIIB) and HIV-1 MN (P18 MN) isolates.

Figure 3
HIV-IT (V)-Induced Anti-gp120 Antibody

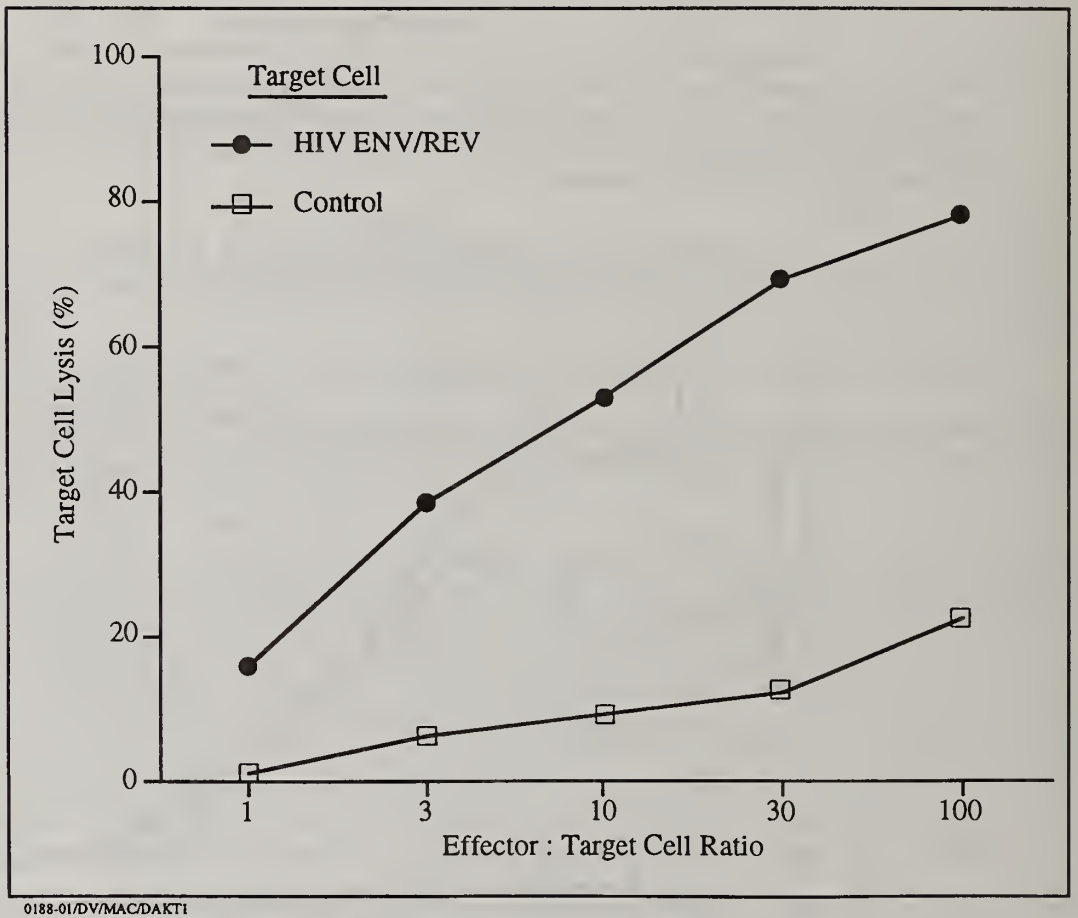


BALB/c mice immunized with HIV-IT (V) produce HIV-1 IIIB gp120-specific antibodies. Mice were immunized IM and their sera tested for binding to purified gp120 protein by ELISA.

2.4.3 Rhesus monkeys have been injected with HIV-IT (V) to evaluate the production of HIV-1 ENV/REV specific CTL in a non-human primate model. The IM administration of one to three doses of HIV-IT (V) (3×10^7 cfu) to Rhesus monkeys has resulted in the development of HIV-1 ENV/REV specific $CD8^+$ MHC restricted CTL responses (Figure 4). Other studies have shown that the cell-mediated cytotoxic response in the monkey has persisted for at least 12 weeks following the last HIV-IT (V) injection. In addition, IM HIV-IT (V) administration to a baboon (2×10^7 cfu) was also shown to induce HIV-1 ENV/REV specific, $CD8^+$, MHC restricted CTL responses and antibody responses. Thus, HIV-IT (V) appears capable of activating cellular and humoral immune responses in non-human primate models.

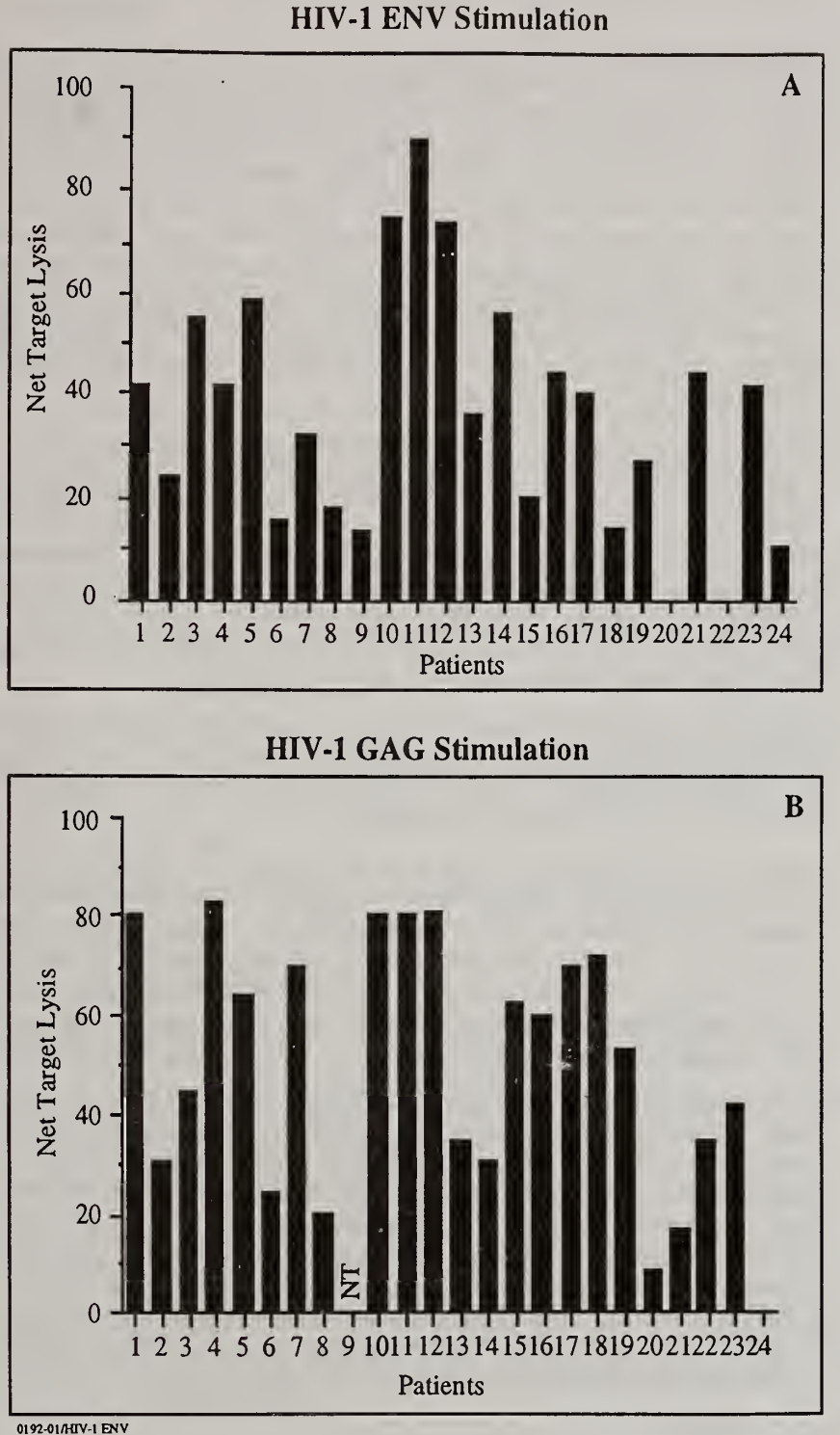
2.4.4 PBMC from HIV-1-infected subjects have been examined to see whether HIV-1 ENV/REV specific CTL responses could be activated using HIV-IT (V)-treated autologous cells. Figure 5 shows that PBMC from HIV-1-infected individuals can be reliably stimulated by HIV-IT (V)-transduced autologous cells expressing the HIV-1 IIIB ENV/REV or HIV-1 IIIB GAG/REV proteins. The stimulated effector cells from a limited number of subjects were characterized as $CD8^+$, Class I MHC restricted CTL.

Figure 4
Rhesus Monkey Cell-Mediated Cytotoxic Activity Following HIV-IT (V) Treatment



Induction of HIV-1 ENV/REV specific T cell-mediated cytotoxic activity in a Rhesus monkey following HIV-IT (V) injection. This monkey was injected three times at two week intervals with a total of 3×10^7 cfu administered per injection in six different IM sites. PBMC were collected prior to each injection and, after *in vitro* stimulation, were tested for cytotoxic activity on HIV-1 ENV/REV expressing or control autologous targets. The data presented employed PBMC taken two weeks after the first injection.

Figure 5
Human Cell Mediated Cytotoxic Activity



CTL activity of HIV-seropositive subject PBMC following *in vitro* stimulation with HIV-IT (V)-transduced autologous cells. Subject PBMC were stimulated *in vitro* with vector-transduced cells expressing HIV-1 IIIB ENV/REV (A) or HIV-1 IIIB GAG/REV (B) proteins. These stimulated effector cells were tested for cytotoxic activity on their respective autologous HIV ENV/REV or HIV GAG/REV targets and control targets. Net target cell lysis is presented. (NT, Not Tested.)

These data support the view that human $CD8^+$ CTL exist in HIV-1-infected individuals and can be stimulated by HIV-IT (V)-transduced autologous cells. It will be important to ascertain whether a similar triggering phenomenon can occur *in vivo*.

2.4.5 The quantity of HIV-IT (V) injected into mice, Rhesus monkeys, and baboons, as well as the projected dose for human clinical studies is very small relative to body cell mass. Due to the non-replicating nature of HIV-IT (V), widespread distribution of the vector after IM injection would not be expected, particularly in the absence of RCR. The majority of HIV-IT (V) should, therefore, remain at the injection site. Although a complete accounting of all HIV-IT (V) material within an injected animal is not feasible with current assays, vector DNA, if present in tissue, should be detectable using sensitive molecular techniques. Two approaches have been used: (1) administration of ^{35}S methionine/cysteine-labeled vector to mice followed by measurement of radioactive counts in various tissues to track vector particles; and (2) polymerase chain reaction (PCR) monitoring of injection sites and testes in mice to track the vector integration of HIV-1 env DNA in the tissues.

The data indicate that the majority of IM administered, ^{35}S-met/cys-labeled vector appears to remain at the site of injection and does not traffic throughout the animal. Further results demonstrate that vector HIV-1 env DNA is detectable by PCR at the site of injection after one or two injections in all mice tested. Results have also indicated that genomic DNA obtained from testes of HIV-IT (V)-injected mice are PCR negative for HIV-1 env DNA. These data suggest that HIV-IT (V) injected IM remains primarily at the injection site until cleared by the immune system and that there is no evidence of vector associated with germline tissue.

2.4.6 In order to determine the acute safety and biological activity of multiple intramuscular injections of HIV-IT (V), a four-week IM toxicity study in male and female BALB/c mice was conducted by Hazelton Laboratories (Vienna, Virginia) in accordance with the Good Laboratory Practice Regulations.

Animals were observed for clinical indications of morbidity and mortality daily, and body weights were recorded weekly. Potential clinical pathology was assessed by hematologic evaluation pre- and post-injection. Gross necropsy and histopathologic evaluation of the injection site, gonads, thymus, mesenteric lymph nodes, marrow, liver, kidneys, brain and any gross lesion in all animals, was performed. The findings of clinical observations, body weights, food consumption, clinical hematology, and necropsy data indicate no evidence of toxicity in mice injected with HIV-IT (V).

2.4.7 In summary, preclinical data support the view that HIV-IT (V) can be safely administered to both mice and non-human primates, that $CD8^+$ CTL and antibody responses can be induced in animal models and that cross-reactive cytotoxic immune responses directed against cells infected with divergent HIV-1-strains can be demonstrated by murine CTL induced by HIV-IT (V). In addition, mouse toxicology studies have found no HIV-IT (V)-related abnormalities. These data provide the impetus to initiate studies of HIV-IT (V) in humans. Based on the successful production of CTL responses achieved in non-human primates that received three doses of HIV-IT (V) and on a careful analysis of these data, Phase I clinical trial subjects will receive three doses of HIV-IT (V) at monthly intervals.

2.5 Clinical Studies

Three successive biweekly injections of autologous fibroblasts transduced with HIV-IT (V) have been administered to each of four asymptomatic HIV-1 infected subjects in a Phase I study conducted at the Shared Medical Research Foundation in Tarzana, California. These subjects have been followed for intervals of from two weeks to two months following their third injection. No product-attributable adverse events have occurred. It is still too early to

ascertain whether meaningful changes will occur in CTL activities, in antibody responses or in viral burden.

3.0 Subject Selection

3.1 Subjects will include up to 25 eligible male and female HIV-1-infected individuals previously or currently enrolled in the CCTG-704 (UCSD 91-786) pilot protocol: "CTL Studies in HIV-1 Infected Subjects Using Retroviral Vector-Transduced Cells." All eligible subjects will receive the immunostimulant HIV-IT (V). Specifically, subjects must meet the following criteria:

3.2 Inclusion Criteria:

3.2.1 Be HIV-1-infected as demonstrated by a Federally licensed positive ELISA Assay for HIV-1 antibodies and confirmed by Western blot analysis.

3.2.2 Be an adult (age 18 to 65).

3.2.3 Have acceptable laboratory values, including hematocrit $\geq$ 34% for men and $\geq$ 30% for women, granulocytes $\geq$ 1500 cells per mm^3, platelet count $\geq$ 100,000 per mm^3, SGOT (AST) $\leq$ 2.5 x the upper limit of normal, SGPT (ALT) $\leq$ 2.5 x the upper limit of normal and creatinine, within normal limits.

3.2.4 Have $CD4^+$ T cell levels $\geq$ 500/µL or $\geq$ 30% and have a positive DTH skin test (Stratum #1) and reside in CDC HIV classification categories A1 or B1 (see CDC HIV Classification from MMWR of 12/18/92 included as Appendix A) or have $CD4^+$ T cell levels from 200-499/µL or 15-29% or have a negative DTH skin test (Stratum #2) and reside in CDC HIV classification categories A2 or B2 (Appendix A).

3.2.5 Female subjects must have a negative serum pregnancy test. Both male and female subjects must use barrier birth control measures adequate to block HIV-1 transmission.

3.2.6 Commit to a 3 year follow-up evaluation schedule in order to properly evaluate safety and biologic activity.

3.2.7 Render an Informed Consent in accordance with institutional policies.

3.3 Exclusion Criteria:

3.3.1 Pregnant or lactating females.

3.3.2 Persons of both sexes, with or without reproductive potential, who do not use barrier birth control measures adequate to prevent HIV-1 transmission.

3.3.3 Subjects with CD4+ T cells levels < 200 or < 15% who reside in CDC HIV classification categories A3, B3 or C (see Appendix A).

3.3.4 Previous treatment with an anti-HIV-1 immunotherapeutic or vaccine.

3.3.5 Recipients of a major organ allograft, or history of significant cardiac, hepatic, pulmonary, reticuloendothelial, renal or central nervous system disease or malignancy.

3.3.6 Previous treatment with anti-neoplastic chemotherapy within six months, experimental therapy within 45 days, or immunomodulators or corticosteroids within four weeks or other medications which might interact with or be contraindicated in association with HIV-IT (V) administration.

3.3.7 The clinical inadvisability of, or participant's unwillingness to suspend anti-viral and/or anti-retroviral therapy for a period three days before and three days after each scheduled dosing of HIV-IT (V).

4.0 Procedures

4.1 Prior to enrollment, subjects will have a complete medical history and physical exam, chest X-ray and EKG as well as routine laboratory testing (CBC, platelet count, chemistry panel, urinalysis). In addition, up to a 120 ml blood sample will be drawn to assess baseline values of potential surrogate markers which will include CTL activity, CD4 and CD8 counts, HIV-1 gp120 antibody titer and leukocyte viral load which will be determined by a standardized PCR-based assay of cellular DNA. If it has not already been performed or has been performed more than one month prior to first injection, a delayed-type hypersensitivity (DTH) skin test will be administered intradermally to assess the degree of cell-mediated immunity. Early signs and symptoms of disease, e.g., CDC HIV classification conditions listed in Category B (see Appendix A) will be assessed by history and physical examination.

4.2 Subjects will be subdivided into two strata based on CDC HIV classification criteria (see Section 3.2.4 above and Appendix A). Each stratum will have approximately half of all subjects in the study. Early safety data (day 90) from the first six subjects in Stratum #1 ($CD4^+$ T cell levels $\geq 500/\mu L$ or $\geq 30\%$) or equivalent data from another study will be made available for IRB review and approval prior to treatment of any stratum #2 subjects ($CD4^+$ T cell levels from 200-499/μL or 15-29%).

4.3 Following lyophilized product reconstitution, each eligible subject will receive a dose of 10^7 cfu on each of 3 injection days spaced at 28 day intervals. Total dose volume may vary, but will not exceed 2.0 ml. A given dose will be delivered in from one to four IM injections made unilaterally or bilaterally into the deltoid and gluteus maximus muscles. If no severe product-attributable toxicities have occurred in a subject within six months of the third HIV-IT (V) administration, up to a full three dose course comparable to the initial treatment course described above may be repeated later in that subject.

4.4 The subject will be observed continuously for the first fifteen minutes following each injection and at 30, 60, and 120 minutes thereafter. Vital signs (BP, P, T, R) will be obtained prior to each injection and at 15, 30, 60 and 120 minutes following each injection.

4.5 Approximately four days prior to the first injection day (#1) and 14 days after each injection, subjects will undergo routine laboratory testing to determine whether it is clinically safe to proceed with treatment. On all injection days, an interim history and relevant physical exam will be performed prior to dose administration to assess clinical status, and to determine whether any toxicities have occurred which preclude further product administration. Injection schedules may vary by $\pm$ 1 day and laboratory testing schedules may vary by $\pm$ 3 days.

4.6 Subjects will return for follow-up at 70 days and at 3, 4, 5, 6 and 12 months following the first injection. Long term follow-up will be conducted at 6 month intervals thereafter for at least three years. Follow-up visits after day 70 may vary by $\pm$ 7 days. Follow-up measures will consist of interim health reports or complete history and physical exams, routine laboratory analyses, CTL activity, CD4 and CD8 counts, HIV-1 gp120 antibody titer, leukocyte viral load, and the DTH skin test (see Subject Study Schedule, Section 11.0).

4.7 The total volume of blood drawn for the first seven visits (up to day 70) will be approximately 430 ml. The total volume drawn from visits 8 through 12 (months 3-12) will be approximately 505 ml. A total of approximately 442 ml of blood will be drawn during visit 13 through 16 (18 months through year 3). No more than 145 ml of blood will be drawn at any one visit.

4.8 Refer to Study Plan, Table 1 and the Subject Study Schedule, Section 11.0, for the timing and frequency of subject evaluations.

5.0 Risks and Discomforts

Risks associated with blood drawing may include transient pain, bruising or lightheadedness, and a rare risk of local infection. Risks associated with DTH testing may include local itching, redness, pain, blister formation, ulceration or necrosis. Very rarely a more serious systemic

allergic reaction may occur. Risks associated with product injections include local pain, inflammation, erythema, swelling, itching, tenderness, skin discoloration, skin breakdown, regional lymphadenopathy, and possibly even granuloma formation at the injection site. Serious inflammation is not anticipated; however, should this occur, symptomatic treatment will be promptly provided. Other clinical manifestations that could result from product administration include fever and chills, diaphoresis, gastrointestinal distress, allergic reactions which may include rash, decrease in blood pressure, shortness of breath and/or syncope, and remotely, anaphylaxis.

There are several theoretical safety concerns related to the use of retrovirally vector-mediated gene transfer[14]. These are summarized in Section 5.1 through 5.5.

5.1 Production of Replication-Competent Virus Through Recombination

The risk of producing replication-competent retrovirus through recombination is remote because (1) All known human endogenous retroviruses have multiple disruptions in their coding sequences[15], and Southern blot analysis of human genomic DNA, probed with the HIV-1 *env* gene, has failed to demonstrate any significant homology; (2) The canine packaging cell utilized in HIV-IT (V) production has been engineered to minimize the possibility of generating replication-competent retrovirus; (3) Due to the split genome approach to production of the packaging cell line, it can be predicted that three separate recombination events would be required in order to produce a replication-competent virus[16]; (4) A minimal number of cell doublings will take place in the manufacturing process; and (5) Quality Control testing using the *Mus dunni* cocultivation procedure and the S^+L^- extended assay to detect amphotropic and xenotropic retroviruses will be performed on HIV-IT (V) prior to Quality Control release for injection.[17]

5.2 Malignancy Caused by Insertional Mutagenesis

From a theoretical standpoint, approximately four to five specific mutations are likely required within a cell to cause malignant transformation[18]. The risk of potential malignant transformation has been assessed by tumorigenicity studies conducted in nude mice and immunosuppressed suckling mice, and by transformation studies in soft agar using cells expressing the HIV-1 IIIB*env/rev* retroviral construct. No evidence of tumor formation in nude and suckling mice or transformation in soft agar using fibroblasts expressing the HIV-1 ENV/REV proteins has been observed.

5.3 Contamination and/or Mutation of Germ Cells

5.3.1 This risk of germ line transmission is viewed as unlikely due to the non-replicating nature of the vector. Further, PCR analyses of sperm samples from male Rhesus monkeys and HIV-1-infected chimpanzees after treatment with autologous cells expressing the HIV-1 IIIB*env/rev* retroviral construct have shown no indication of transfer of HIV-IT (V) into the germ line tissues of these animals. Additional studies in mice have detected no evidence of HIV-1 DNA in testicular tissue taken from mice injected with retroviral vector bearing the HIV-1 IIIB*env/rev* construct.

5.3.2 Although it is considered unlikely based on the above animal studies, HIV-IT (V) does have the theoretical potential to alter germline genes. The risk to a future child is unknown and information from animal studies may or may not predict what will happen in humans. Participants in this study should make every effort to avoid pregnancy (see Sections 3.2.5 and 3.3.2 and Procedures (B) under Informed Consent). If pregnancy occurs, a discussion will be held with the Principal Investigator

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[14]Cornetta K. et al., *Human Gene Therapy*, 2:5-14, 5-15, (1991).

[15]Abraham, G.N. and Khan, A.S., *Clin. Immunol. & Immunopath.*, 56:1-8 (1990).

[16]Miller, D.A., *Human Gene Therapy*, 1:5-14 (1990).

[17]Bassin, R.H., et al., *Int. J. Cancer*, 6:95-107 (1970).

[18]Fearon, E., and Vogelstein, B., *Cell*, 61:759-67 (1990).

concerning the option of continuing pregnancy.

5.4 Further Immune Suppression Precipitated by the Release of Active Infectious HIV-1 Virus From CTL-Lysed HIV-Infected Macrophages

5.4.1 The magnitude of this risk is uncertain, however, most intracellular HIV-1 is not infectious and becomes infectious only after budding from infected cell membranes.

5.4.2 Clinical and laboratory parameters will be monitored for evidence of exacerbation of disease. See study schedule, Section 11.

5.4.3 $CD4^+$ T-cells will be monitored in samples taken prior to each injection and at each visit thereafter. Viral burden will also be monitored (see Study Schedule, Section 11.0).

5.5 Exacerbation of Disease Due to the Killing of Uninfected CD4 T-Cells as a Result of HIV-1 IIIB ENV Toxicity

The magnitude of this risk is uncertain, however, previous vaccine studies, using HIV-1 ENV soluble antigen have not caused any measurable side effects in treated subjects[19]. Moreover, no indications of significant $CD4^+$ T cell instability or other safety related symptoms have occurred in four HIV-1 positive subjects who received a series of three injections of HIV-IT (V)-transduced autologous fibroblasts and have been followed for intervals of from two weeks to two months beyond the third injection.

6.0 Risk Management

6.1 The risks to subjects and health care professionals from the blood drawing will be minimized by careful attention to technique and close subject follow-up. The theoretical risks associated with gene therapy will be minimized by manufacturing practices and procedures and quality control testing.

6.2 Safety will be evaluated by physical examination, interim clinical history, and laboratory analyses. Any deviations from normal will be scored according to the ACTG toxicity scale included in Section 12.0.

6.3 Adverse reactions associated with product administration will be treated symptomatically by the principal investigators. If any subjects experience adverse reactions which are incapacitating or life threatening, the appropriate interventional therapy will be promptly administered. If recurrent grade 3 or greater ACTG toxicity, possibly or probably related to study therapy, is encountered in a subject, further administration of HIV-IT (V) will be suspended in that subject. If repeated grade 3 or greater toxicity, possibly or probably related to study therapy, is encountered in multiple subjects, modifications in this study will be implemented as set forth in Section 6.5 below.

6.4 The Investigators will furnish the Sponsor with Safety Reports after subjects have completed their visit 2 weeks after the third injection, at 6 months and after their 1, 2 and 3 year follow-up visits. If subjects develop symptoms which justify further diagnostic tests and/or procedures, results of such tests and/or procedures should be reported in appropriate Case Report Forms. Such Case Report Forms will be submitted to Viagene with the Safety Reports even if test results are still pending. Any follow-up test/procedure information should be forwarded to Viagene as soon as results are available.

6.5 Impact of Adverse Reactions on Study Plan: Dosage Modification Schedule

6.5.1 If recurrent grade 3 or greater ACTG toxicity, possibly or probably related to study therapy, (see Section 12.0) is observed in four subjects receiving 10^7 cfu of HIV-IT (V), the remainder of the subjects will receive a lower dose of 10^6 cfu. If recurrent grade 3 or greater ACTG toxicity, possibly or probably related to study therapy, is observed in three subjects receiving the lower dose, this study will be suspended.

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[19]Redfield R.R. et al., *N. E. Journal of Med.*, 324:1677-1684 (1991).

6.6 Conditions Which May Necessitate Discontinuation of a Subject's Treatment

6.6.1 If recurrent ACTG grade 3 or greater toxicity, possibly or probably related to study therapy, is encountered; or

6.6.2 If, in stratum #2, subjects with entry $CD4^+$ T cell counts from 200-499/μL or from 15-29% repeated $CD4^+$ T-cell counts obtained at least 72 hours apart decline to < 50% of pre-treatment values.

6.6.3 If, in the attending physician's judgment, the subject's condition precludes even temporary suspension of approved anti-retroviral drug therapy; or

6.6.4 If there are any willful protocol violations; or

6.6.5 If a subject develops another illness which requires treatment with a drug or agent (e.g., any immunosuppressive or chemotherapeutic agent) which is likely to interfere with the activity of the gene-based immunostimulatory product investigated under this protocol; or

6.6.6 If the subject elects to withdraw from the study; or

6.6.7 If, in the opinion of the attending physician, it is in the subject's best interest to withdraw from the study.

6.7 Termination of Study and Follow-Up Evaluation

6.7.1 Product administration may be terminated at any time, at the subject's request.

6.7.2 Subject participation may be terminated by the attending physician if justified by the subject's clinical status or if deemed in the subject's best interest.

6.7.3 The protocol or any individual subject's study may be terminated by written communication from Viagene.

6.7.4 Anyone receiving experimental treatment will be followed for at least 3 years.

7.0 Potential Benefits

Society and those infected with HIV-1 will benefit if this therapy is tolerated and proves to be effective in larger trials. HIV-IT (V) has the theoretical possibility of generating an increased immune response, particularly $CD8^+$ CTL activation, which may retard HIV-1 spread and progression towards AIDS.

8.0 Benefit/Risk Ratio

The benefit/risk ratio is justifiable because many precautions are taken to minimize the risk associated with the HIV-IT (V) product for recipient subjects. There is the possibility that HIV-IT (V) may stimulate an immune response that may help control HIV-1 disease progression.

9.0 Plan of Analysis

9.1 Data collected during this Phase I/II study will include the following variables: clinical history and physical examination parameters, vital signs, report of adverse events, chemistry profile, CBC and differential, platelet count, urinalysis, $CD4^+$ T-cell count, HIV-1 antibody titer, leukocyte viral load by PCR and CTL response. These variables will be measured at multiple time periods. Findings from this study will be tabulated and presented, and results will be analyzed. All out of range laboratory data will be addressed.

9.2 Pre-treatment versus post-treatment comparisons of selected continuous variables will be made using analyses of variance (ANOVAs). Pre-treatment versus post-treatment comparisons of selected categorical variables will be made using Fisher's Exact tests or categorical log-linear models.

9.3 Upper 95% exact binomial confidence limits will be performed for any toxicities whic...

occur. These limits will be used to predict the maximum toxicity rate which might be expected to occur in the larger population of the same subject subset.

The table below illustrates this analysis. For example, if a single ACTG Toxicity Scale Grade I or greater toxic event is observed within ten subjects, then, with a power equal to 80% and an alpha of 5% (one-sided), the true, expected toxicity rate in the larger population would be unlikely to exceed 40%.

Upper 95% exact binomial confidence limits:

Number of Subjects	Percent of subjects likely to experience a Grade I or greater toxic reaction when number of observed reactions is		
	0	1	2
5	45	66	82
10	26	40	51
15	19	28	37

10.0 Consent Procedures

Subjects will receive a copy of the "Patient Brochure", fully describing the nature of the vector, the purpose of the study, and its design, including the relevant background and research findings, a comprehensive description of dose administration, and attendant risks, discomforts, and potential benefits. After this brochure has been reviewed by the subject and discussed with the Principal Investigator, informed consent will be obtained by the investigators prior to initiating the protocol. The Informed Consent follows in Appendix 1.

11.0 Appendices

Appendix 1: Informed Consent

Appendix 2: CDC HIV Classification (MMWR 12/18/92)

Appendix 3: Table 1 — Study Plan

Appendix 4: CCTG Study Schedule

Appendix 5: ACTG Toxicity Scale

HUMAN SUBJECTS COMMITTEE
Approved
6-3-93
Date 8-5-93

UCSD Project 93-418
7/22/93
CCTG 705
Version 1.4

UNIVERSITY OF CALIFORNIA - SAN DIEGO
CONSENT TO ACT AS A RESEARCH SUBJECT

CCTG 705: AN OPEN-LABEL, PHASE I/II CLINICAL TRIAL TO EVALUATE THE SAFETY AND BIOLOGICAL ACTIVITY OF HIV-IT (V) (HIV-1 III_B ENV/REV-RETROVIRAL VECTOR) IN HIV-1 INFECTED SUBJECTS.

Drs. Richard Haubrich, J. Allen McCutchan, Diane Havlir, T.C. Meng, Susan Little, and their associates are conducting a study to see if immunization with HIV-IT (V) can improve my body's ability to produce killer cells against HIV. Part of the normal immune response to HIV is to produce CTL (cytotoxic T lymphocytes) - which are cells that kill HIV. Preliminary studies in mice and monkeys have shown that HIV-IT (V) can stimulate CTL in these animals. The purpose of this study is to evaluate the safety of HIV-IT (V) and to see if it increases the level of CTL in my body. I have been asked to participate because I have HIV infection.

If I agree to participate the following will happen:

1. I will be seen for a screening evaluation which includes:

   A. A medical history and physical exam.
   B. Blood tests, including a pregnancy test (if applicable)
   C. Skin testing.

The skin tests are done by injecting, using a very small needle, standard skin test substances into my arm. I will return in two days to have this skin test read. In addition to standard laboratory tests I will have 120 ml of blood (about 1/2 cup) drawn to evaluate CTL in my blood. If I qualify, then I will return to the clinic for injection of HIV-IT (V). I must stop my anti-HIV medication (i.e. AZT, ddI ,or ddC) three days before each injection and remain off therapy until three days after each injection. I will receive three monthly injections of HIV-IT (V) into a muscle in an arm, the buttocks, or both. After each injection, I will be observed in the clinic for two hours to look for side effects.

Two weeks after each injection, I will return to the clinic for a safety evaluation which includes history, relevant physical exam and blood tests to monitor safety and to look for CTL. If I am female, a repeat pregnancy test will be run on days 14 and 42.

I will return to the clinical at months 3, 4, 5, 6, 12, 18, 24, 30, and 36 and have similar evaluations to ensure safety and to look for the effect of the injections on my CTL's.

Participation may involve some added risks or discomforts. These include:

A. The risk of drawing blood includes pain, bruising, and rarely, local infection.
B. The risk of skin testing includes pain, itching, redness, blisters, and very rarely, an allergic reaction.
C. Injection with HIV-IT(V) may cause pain, soreness, redness, warmth, itching, numbness or swelling at the site of injection.
D. Rarely an allergic reaction to any of the material injected could occur. If this happens fever, chills, sweating, dizziness, shortness of breath, rash, itching, nausea, vomiting, or low blood pressure might occur. Very rarely an individual may experience a life threatening allergic reaction to any material.
E. The risks of injection of HIV-IT (V) are unknown at this point. Potential risks include the following:

   i. Disease could be caused by the disabled virus which is used to insert a new gene into my cells.
   ii. Unknown disease could be caused by the genetic material activating an unknown virus in my body.
   iii. Effects could occur from undetected contaminants from the treatment.
   iv. Cancer or lymphoma could occur.
   v. HIV infection may be made worse by this therapy.

F. HIV-IT(V) has a theoretical risk of changing the genes of my sperm or ova. This is considered unlikely because animal experiments have shown no evidence that HIV-IT(V) can change the genes in the sperm of the animals tested. **However, the risk to a future child is unknown and information from animal studies may or may not predict what will happen in humans.**

G. The above complications were not observed in animal studies using HIV-IT(V). The viral vector (HIV-IT(V)) will be tested for safety before I am injected to reduce any risk of it causing illness or side effects. My CD4 cell count and other blood tests will be checked to assure that no health problems have occurred. If my CD4 count or other lab tests suggest that the treatment is making my HIV infection worse, then I will not be given further injections.

H. Participation in the study requires that I use a barrier form of contraception (ie condom). This is to prevent 1) pregnancy in myself or my partner and 2) the possibility of transmitting the disabled virus in HIV-IT(V).

I. If I am female and capable of child bearing, I will have a pregnancy test prior to beginning the study in order to ensure that I am not pregnant. During the study, I should make every effort to avoid pregnancy. If I miss a period or think I might be pregnant, I will notify the study doctor immediately. If I become pregnant, study therapy will be discontinued. If I become pregnant I may discuss with my doctor the option of continuing the pregnancy.

J. No more than 145 ml (5 ounces) of blood will be drawn at any on visit. The total amount of blood that will be drawn is 430 ml (15 ounces) during the first 70 days of the study. 505 ml (18 ounces) will be drawn during months 3 to 12. 442 ml (15 ounces) will be drawn during years 2 and 3.

There is some risk in not taking my anti-HIV medication for the total of 18 days. The exact risk of this is unknown, although during this time the HIV in my body could increase.

If I am injured as a result of participation in this research, the University of California will provide any medical care I need to treat those injuries--except when they are a consequence of research procedures designed to benefit me directly. The University will not provide any other form of compensation to me if I am injured. I may call (619) 534-4520 for more information about this or to report research-related problems.

There may be no direct benefit to me from this treatment. It is possible that my immune system may produce increased levels of CTL against HIV. Information from this study will help investigators learn more about the immune defenses to HIV and whether or not we can increase these defenses with HIV-IT (V). There is no known alternative to this therapy at this time. If my T-cell count is above 500, currently there is no indication for therapy. If my T-cell count is less than 500, I can receive standard therapy with AZT, ddI, ddC, or no therapy at all.

_____ has explained the study to me and has answered my questions. If I have other questions or research related problems I may reach Dr. Richard Haubrich at 543-8080.

Participation in research is entirely voluntary. I may refuse to participate or withdraw at any time without jeopardy to the medical care I will receive at this institution.

Research records will be kept confidential to the extent provided by law. Records of this research will not contain my name. Representatives from Viagene, Inc., or the FDA may look at these records to monitor safety. These research records or any publication resulting from the study will not use my name.

I have received a copy of the consent document to keep and a copy of "The Experimental Subject's Bill of Rights".

I agree to participate.

Subject's Signature	Date
Witness	Date

UCSD Project 93-418
7/22/93
CCTG 705
Version 1.4

SAN DIEGO VETERAN'S ADMINISTRATION MEDICAL CENTER
CONSENT TO ACT AS A RESEARCH SUBJECT

CCTG 705: AN OPEN-LABEL, PHASE I/II CLINICAL TRIAL TO EVALUATE THE SAFETY AND BIOLOGICAL ACTIVITY OF HIV-IT (V) (HIV-1 III_B ENV/REV-RETROVIRAL VECTOR) IN HIV-1 INFECTED SUBJECTS.

Drs. Richard Haubrich, J. Allen McCutchan, Diane Havlir, T.C. Meng, Susan Little, and their associates are conducting a study to see if immunization with HIV-IT (V) can improve my body's ability to produce killer cells against HIV. Part of the normal immune response to HIV is to produce CTL (cytotoxic T lymphocytes) - which are cells that kill HIV. Preliminary studies in mice and monkeys have shown that HIV-IT (V) can stimulate CTL in these animals. The purpose of this study is to evaluate the safety of HIV-IT (V) and to see if it increases the level of CTL in my body. I have been asked to participate because I have HIV infection.

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Participation may involve some added risks or discomforts. These include:

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E. The risks of injection of HIV-IT (V) are unknown at this point. Potential risks include the following:

   i. Disease could be caused by the disabled virus which is used to insert a new gene into my cells.
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There is some risk in <u>not</u> taking my anti-HIV medication for the total of 18 days. The exact risk of this is unknown, although during this time the HIV in my body could increase.

If I am injured as a result of participation in this research, treatment will be available. If I am eligible for veteran's benefits, the cost of such treatment will be covered by the Veteran's Administration. If not, the cost of such treatment may be covered by the Veteran's Administration and the University of California, depending on a number of factor. The Veteran's Administration and the University of California do not normally provide any other form of compensation for injury. For further information about this, I may call tha VA District Counsel at (619) 557-6718 or the Human Subject's Committee Office at (619) 534-4520.

There may be no direct benefit to me from this treatment. It is possible that my immune system may produce increased levels of CTL against HIV. Information from this study will help investigators learn more about the immune defenses to HIV and whether or not we can increase these defenses with HIV-IT (V). There is no known alternative to this therapy at this time. If my T-cell count is above 500, currently there is no indication for therapy. If my T-cell count is less than 500, I can receive standard therapy with AZT, ddI, ddC, or no therapy at all.

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Participation in research is entirely voluntary. I may refuse to participate or withdraw at any time without jeopardy to the medical care I will receive at this institution.

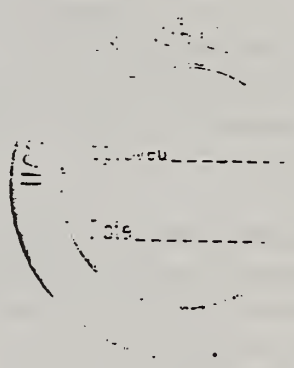
Research records will be kept confidential to the extent provided by law. Records of this research will not contain my name. Representatives from Viagene, Inc., or the FDA may look at these records to monitor safety. These research records or any publication resulting from the study will not use my name.

I have received a copy of the consent document to keep and a copy of "The Experimental Subject's Bill of Rights".

I agree to participate.

_____ _____
Subject's Signature Date

_____ _____
Witness Date



BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Richard H. Haubrich, M.D.	**Co-Investigator**

EDUCTION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Occidental College, Los Angeles, CA	B.A	1979	Chemistry
USC School of Medicine, Los Angeles, CA	M.D.	1983	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

RESEARCH AND PROFESSIONAL EXPERIENCE

1987 - 1990 Fellow, University of Massachusetts Medical Center, Worcester, MA

1990 - 1991 Assistant Professor of Medicine, Department of Medicine, University of Massachusetts Medical Center, Worchester, MA

1991 - Pres. Assistant Clinical Professor of Medicine, Department of Medicine, UCSD, San Diego, CA

HONORS

1990 Maxwell Finland Young Investigators Award

PUBLICATIONS

1. Swendlow, N., and Haubrich, R. (1987). "Aggressive treatment of a patient with the acquired immune deficiency syndrome." *(letter) Annals of Internal Medicine.* 107:597.

2. Haubrich, R.H., Tukeda, A., Koff, W., Smith, C., and Ennis, F.A. "Lack of FCR mediated enhancement in sera from recipients of RGP160 experimental HIV vaccine." *VII Int'l. Confr. AIDS.* Abstract MA 1327.

HUMAN GENE TRANSFER PROTOCOL

9312-063 Sznol, Mario; National Institutes of Health, Frederick, Maryland; *A Phase I Trial of B7-Transfected Lethally Irradiated Allogeneic Melanoma Cell Lines to Induce Cell Mediated Immunity Against Tumor-Associated Antigens Presented by HLA-A2 or HLA-A1 in Patients with Stage IV Melanoma.*

Date of RAC Approval: 12/3/1993
Date of NIH Approval: 4/19/1994

Technical Abstract

A phase I trial of B7-transfected allogeneic melanoma cell lines to induce cell-mediated immunity against tumor-associated antigens presented by HLA-A2 or HLA-A1 in patients with stage IV melanoma.

BRMP 9401
NCI Protocol T93-0161

Biological Response Modifiers Program, DCT, NCI

The scientific basis for this protocol is derived from two main points. Recent data indicate that two stimuli are required to activate T cells to proliferate and secrete cytokines. Signal one is provided by the T cell receptor, and the other requires interaction of other T cell surface receptors with their ligants on antigen presenting cells. The second point, is that in the case of human melanoma, tumors from many patients express the same tumor antigens, and that peptides derived from these tumor antigens are often presented on tumor cell surfaces by MHC class I molecules HLA-AI or A2. Thus, it may be possible to induce immunity against these shared melanoma antigens by vaccination with allogeneic tumor cell lines that express the tumor antigens and are HLA-A1 or HLA-A2 positive. In order to study these questions in a clinical trial of advanced stage melanoma patients, we introduced the gene encoding the T cell co-stimulatory molecule B7 (CD28 ligand) into 3 human melanoma cell lines. These tumor lines are HLA-A2+ (one is HLA-A1+), express significant levels of LFA-3 and ICAM-I (and in one case HLA-DR) on their surfaces, and produce RNAs encoding the shared melanoma antigens MAGE-I and -3, and tyrosinase. We have examined the stimulatory capacity of parental tumor lines and B7-transfectants (DM150/B7-8, DM13/B7-7, and DM93/B7-4) in 7 day primary mixed lymphocyte cultures (MLC) with allogeneic human T cells obtained from PBL of normal donors or melanoma patients. In multiple experiments, parental tumor lines (which do not express detectable B7) fail to induce significant activation of allogeneic T cells as determined by lack of increased expression of HLA-DR or CD25 on T cell surfaces. In contrast, B7-transfected lines induce increased expression of HLA-DR and CD25 (in both CD4+ and CD8+ subsets), and a 5-10 fold increase in T cell number compared to cultures with parental cell lines. CTL induction in primary MLC was determined in this system by 7d co-culture of allogeneic T cells with the parental DM150 or DM150-B7 cell lines. While T cells cultured with DM150 exhibit only background levels of lytic activity, T cells cultured with DM150-B7 lyse this line, and also the unmodified parental line and the HLA-A2+ DM13 cell line. TIL lines that recognize the shared melanoma antigen(s) presented by HLA-A2 lyse all three parental lines, providing functional data that these lines present shared melanoma antigens via the endogenous MHC class I pathway. These data indicate: 1. B7 is expressed by all three lines and is biologically functional as assessed by the ability to activate resting human T cells. 2. The cell lines express the genes encoding the known shared melanoma antigens and are lysed by HLA-A2 specific TIL derived from melanoma patients, demonstrating functional expression of the shared melanoma antigens by these lines. These studies form the preclinical basis for a vaccine trial in which patients will be vaccinated with lethally irradiated allogeneic melanoma cell lines genetically engineered to express human B7. The three lines will be injected subcutaneously at two week intervals for three vaccinations, followed by 3 injections at monthly intervals. The cell lines will be given on a rotating basis. Cohorts of patients will receive escalating doses of 10^7, 10^8, or 10^9 cells. This is a Phase I trial to determine the MTD of this therapy, but immunologic parameters such as generation of CTL precursors in peripheral blood and draining lymph nodes will also be monitored.

Non-Technical Abstract - BRMP 9401

A phase I trial of B7-transfected allogeneic melanoma cell lines to induce cell-mediated immunity against tumor-associated antigens presented by HLA-A2 or HLA-A1 in patients with stage IV melanoma.

BRMP 9401
NCI Protocol T93-0161

Biological Response Modifiers Program, DCT, NCI

It is possible for the body's immune system to recognize and reject some cancers. This probably occurs when blood immune cells called lymphocytes recognize an abnormal protein (called an antigen) on the tumor cell, attach to the tumor, and then either kill the tumor or call other immune cells to the site to help eliminate the cancer cells. Scientists have recently discovered that the tumors from some patients with melanoma contain distinct antigens that can be recognized by lymphocytes. However, for reasons that we still do not understand, in most patients with melanoma the immune system has somehow been paralyzed and cannot recognize or attack the tumor.

It may be possible to activate the immune system to recognize the antigens on melanoma cells. We now understand that if lymphocytes see an antigen on a tumor cell but don't receive an important second signal (which is not normally provided by the melanoma cell), the lymphocyte will be turned off. We have grown tumor cell lines in the laboratory that contain some of the melanoma antigens. We have taken a gene (a gene is the DNA that directs the production of a protein in a cell) which codes for a molecule called B7 and placed it in the melanoma tumor cell lines we have in the laboratory. We believe that the B7 will provide a necessary second signal to activate lymphocytes to recognize the melanoma tumor antigens. Once activated, the lymphocytes can recognize and kill other melanoma cells even if they do not express the B7 signal.

In order to administer treatment, we match the patient to the tumor cell lines we have in the laboratory. We do this by determining the expression of specific proteins on the surface of patient lymphocytes that we obtain prior to the study. Only patients whose lymphocytes express the proteins HLA-A1 or HLA-A2 can receive treatment on this study.

For treatment, we plan to inject a large number of cells from the tumor cell lines containing B7 under the skin. The cells are radiated so there is little chance that a tumor might grow where we inject the cells. The tumor cells are injected every two weeks for 3 doses, then once a month for 3 doses. We rotate the sites of injection, usually starting in the leg, then the arm, then the opposite arm, then the opposite leg, and so on. HLA-A1 patients receive the same dose of the same cell line each time. HLA-A2 patients will alternately receive one of three cell lines. In either group, a maximum of 6 treatments is planned.

We will treat groups of patients with each group receiving a larger number of cells. The purpose of the study is to determine how many cells we can give safely and whether an immune response develops to the cells as we predict from the laboratory studies. In the first or second group of patients, 2 injections are given (about 2 inches apart) each treatment. In the third group, 10 injections are given each time. We do not know if the B7 actually improves the immune response to the cells. Therefore, the last group will receive the same tumor cell line(s) but not containing B7. If patients do not have a good response after receiving all 6 planned injections, we may try to give the cells containing B7 in the same way as long as the patient is still in good enough condition to continue treatment on this protocol.

A Phase I trial of B7-transfected lethally irradiated allogeneic melanoma cell lines to induce cell-mediated immunity against tumor-associated antigen presented by HLA-A2 or HLA-A1 in patients with stage IV melanoma

NCI Protocol T93-0161
BRMP Protocol 9401

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October 6, 1993 3:00pm

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I. Introduction

In 1991, approximately 32,000 new cases of melanoma and 6500 deaths from metastatic melanoma were reported in the United States (reviewed, 1). The incidence has been increasing steadily in the last several decades, and by the year 2000, it is estimated that 1 in 90 individuals may develop melanoma during their lifetime. Although most lesions are curable by surgical resection at the time of diagnosis, if the disease metastasizes to regional lymph nodes or beyond, the prognosis for long-term survival is poor. Patients with metastases beyond the regional lymph nodes generally have a median survival of less than 12 months and 5-year survival rates of approximately 5%. Unfortunately, treatment for metastatic melanoma has been ineffective. DTIC and cisplatin are probably the most active single chemotherapeutic agents with objective response rates between 10-20%. Although some combination chemotherapy regimens produce response rates up to 50%, most of the responses are partial rather than complete and have a median duration of less than 6 months. No treatment regimen appears to have prolonged median survival for patients with metastatic disease. In order to improve the outcome of patients with metastatic melanoma, novel approaches will be required. A current area of intense investigation is the application of agents that enhance host-mediated immune responses directed against this tumor.

The potential for developing successful immunotherapies for melanoma has been demonstrated in preclinical and clinical studies of interleukin-2 (IL-2). IL-2 was shown to activate peripheral blood lymphocytes (PBL) in vitro, producing a population of cells called lymphokine-activated killer cells (LAK) that were able to kill both autologous and allogeneic fresh tumors and tumor cell lines (reviewed, 2). The subset of lymphocytes with lytic capacity was primarily derived from natural killer precursors, expressed the CD56 antigen, and had minimal lytic activity against normal cells. When IL-2 was administered with LAK cells to tumor-bearing mice, the combination demonstrated potent antitumor activity. Based on these data, clinical trials of IL-2 and LAK cells were initiated in patients with metastatic melanoma, resulting in a few durable remissions and overall response rates in the range of 15-20% (3,4). Subsequent studies showed that high doses of IL-2 alone could produce similar response rates (5,6,7). The results of these studies confirmed that an agent such as IL-2 could produce tumor regression in some patients with advanced disease.

In order to understand the reasons for treatment failure in the majority of patients, attempts were made to further define the mechanisms responsible for IL-2's antitumor activity in vivo. Although endogenous or adoptively

transferred NK/LAK cells were thought to play a major role in mediating the antitumor effects of IL-2, CD56+ cells (the NK/LAK phenotype) were not found in biopsies of responding lesions from patients with metastatic melanoma (8). Selective lymphocyte depletion experiments in a variety of murine tumor models demonstrated that T lymphocytes (particularly CD8 cytotoxic T lymphocytes (CTL)), and not NK/LAK cells, were responsible for the tumor regression observed when IL-2 was administered to the tumor-bearing animals. Rosenberg et al subsequently demonstrated that tumor antigen-specific CTL could be derived from ex vivo cultures of tumor biopsies supplemented with IL-2 (also called tumor-infiltrating lymphocytes or TIL) (9). TIL had more potent antitumor activity than LAK in the animal models by a factor of approximately 100.

On the basis of these data, and evidence that autologous tumor-specific TIL could be expanded from tumor biopsies of patients with metastatic melanoma, clinical trials of IL-2 in combination with TIL were initiated. To date, IL-2/TIL has been reported to produce response rates of 35-40% (3,10), although the contribution of TIL to the activity of IL-2 has not been demonstrated in randomized studies. One interesting observation has been that some patients failing IL-2 alone or with LAK will respond to IL-2/TIL. The importance of these antigen-specific CTL in producing tumor regression is further supported by evidence that response to IL-2/TIL is correlated to both specific cytotoxicity of the TIL cells for autologous tumor in vitro, and to the number of TIL cells administered to the patient (10,11).

The clinical and preclinical studies of TIL indicate that induction or adoptive transfer of antigen-specific T cells can lead to tumor regression in vivo, and that antitumor effects are cell dose-related. In the clinical studies of TIL conducted to date, the ability to expand TIL in vitro has been limited. Furthermore, even when large cell doses are administered, many of the cells may not be specific for the relevant tumor antigen. It may be possible to more effectively induce or expand the antigen-specific response to melanoma, which may result in higher response rates or improved quality of tumor responses in patients. To accomplish this goal, we believe it may be necessary to first induce a strong CTL response in vivo, followed by isolation and expansion of the melanoma-specific CTL in vitro, followed by repeated adoptive transfer of large numbers of the CTL to patients.

The goal of the first generation of our clinical studies is to induce a strong melanoma-specific CTL response in vivo, by immunizing with genetically modified lethally irradiated melanoma cell lines containing shared melanoma-associated antigens. The modifications are intended to increase the

immunogenicity of the cell lines. It is interesting to note that vaccine studies have been conducted for years, using either autologous tumor or allogeneic tumor cell lines admixed with BCG, or lysates of allogeneic melanoma cell lines combined with strong immunologic adjuvants. Regression of advanced disease has been observed in a small number of patients, and at least one group is claiming an improvement in survival of advanced stage patients who were immunized with a panel of allogeneic melanoma cell lines and BCG (12). Unfortunately, these claims are made on the basis of comparisons to matched historical control groups; to be certain of efficacy, the results must be confirmed in randomized controlled trials. Nevertheless, the previous efforts to administer `therapeutic' vaccines provide evidence that active immunization alone may have some antitumor activity in metastatic melanoma.

The melanoma tumor vaccine study we plan to conduct is based on the following principles and recent developments, which are explained in greater detail below:

1. Melanomas from a subset of patients express the same tumor-related antigen(s). Distinct antigens are presented by HLA-A2.1 (subsequently referred to as HLA-A2) and HLA-A1 and are recognized by the host T cell response. Therefore, immunizations can be attempted with allogeneic melanoma cell lines that express the melanoma antigen(s) and HLA-class I phenotype of the patient's tumor, and in vivo T cell responses to immunization can be measured against melanoma cell lines that also express the common antigen(s) and HLA class I type.

2. Effective antigen presentation to T cells in vivo requires both the crosslinking of the T cell receptor by the peptide/MHC complex and a second costimulatory signal. The costimulatory signal can be generated by an interaction between the molecule B7 on the antigen-presenting cell (APC) and its ligand CD28 on the surface of the T cell. The presence of B7 on the APC induces T cell activation and proliferation, while its absence during antigen presentation can induce T cell tolerance under certain circumstances. The melanoma cell lines used for immunization in this study will be transfected with the human B7 gene to enable them to directly stimulate melanoma antigen-specific T cell responses.

Evidence for a shared melanoma antigens presented by HLA-A2 and HLA-A1

CD8+ cytotoxic T cells derived from TIL or mixed lymphocyte-tumor cultures (MLTC) from HLA-A2 melanoma patients have been shown to kill a majority of HLA-A2 allogeneic melanoma cell lines (13, 14). For example, Crowley et al

have shown that peripheral blood or draining lymph node lymphocytes taken from an HLA-A2 melanoma patient can be cultured in vitro with an HLA-A2 allogeneic melanoma, resulting in CTL with specific lytic activity against autologous tumor and other allogeneic melanomas bearing HLA-A2 (15). Interestingly, reactivity against the allogeneic HLA determinants on the sensitizing HLA-A2 melanoma does not occur to a significant degree. These data imply that an antigen (or antigens) that is restricted by HLA-A2 is shared among patients with melanoma. The antigen is also present in melanomas of patients that are not HLA-A2 positive, since transfection of the HLA-A2 gene into HLA-A2 negative melanomas renders them susceptible to lysis by HLA-A2 CTL (16). Although CTL have also been generated that kill allogeneic melanomas sharing HLA-A3, HLA-A1, HLA-A11, HLA-A24, HLA-A31, and HLA-Cw7, the antigen(s) presented by HLA-A2 is preferred for vaccine studies since HLA-A2 is expressed by 50% of the caucasian population. At this time the number of distinct melanoma antigens presented by HLA-A2 is not known. At least 6 different peptide fractions have been eluted from HLA-A2 melanoma cell lines that are recognized by HLA-A2 CTL (17). At a recent meeting, Dr. Thierry Boon and colleagues reported that two proteins, tyrosinase and a second undefined 10 kd protein, contain the peptides that are recognized by the HLA-A2 melanoma-specific CTL. Two immunogenic peptide sequences of tyrosinase have been identified. The expression of tyrosinase and the 10 kd protein appear to be restricted to melanocytes and melanomas. Forty of 40 melanoma cell lines examined to date (100%) have been found to express the tyrosinase gene.

Shared melanoma antigens presented by HLA-A1 (present on 25% of the caucasian population) have also been identified and the gene sequence determined. The antigen, MAGE-1, is a normal protein expressed on 40-50% of melanomas and some other tumors but not on most normal tissues (18). Similar to the HLA-A2 system described above, HLA-A1 CTL specific for MAGE-1 expressing melanoma will kill allogeneic melanomas also expressing HLA-A1 and MAGE-1. At a recent meeting, Dr. Boon's group also presented data that a second melanoma-specific antigen belonging to the MAGE family (MAGE-3) can be presented by HLA-A1. MAGE-3 appears to be present on 60-70% of HLA-A1 melanoma patients.

An animal model has been described which demonstrates that HLA-A2 CTL (generated by coculturing peripheral blood lymphocytes from a HLA-A2 positive patient with an HLA-A2 matched allogeneic melanoma) can mediate tumor regression in vivo (19). The CTL developed specific cytolytic activity against autologous and HLA-A2 matched allogeneic melanomas but not against the allogeneic determinants of the stimulator melanoma cell line. The CTL were administered (in combination with low dose IL-2) to nude mice bearing 3, 7, 10, and 14-day liver metastases of a separate allogeneic HLA-A2 matched

melanoma. CTL plus IL-2 had marked therapeutic activity in the 3 and 7 day models, rendering most mice tumor-free. Some 'cures' were still evident even in the advanced 10-day model. Anti-tumor effects of the CTL were dose-related and specific. IL-2 alone was ineffective, and HLA-A11/HLA-A3 CTL (whose lytic specificity for melanoma cell lines is restricted by HLA-A11) had no anti-tumor effects in vivo or in vitro against the HLA-A2 allogeneic melanoma cell line.

Transfection of B7 to increase the immunogenicity of the immunizing melanoma cell line

B7 is a member of the immunoglobulin gene superfamily (reviewed, 20). It is a transmembrane glycoprotein with two immunoglobulin-like extracellular domains and a core molecular mass of 30 kd. B7 binds to two antigens on T lymphocytes, CD28 and CTLA4, the former which is constitutively expressed on 95% of CD4+ T cells and 50% of CD8+ T cells, and the latter whose expression is induced upon T cell activation. The interaction between B7 on the APC and its ligand on the T lymphocyte markedly enhances T cell activation and production of IL-2. Under some experimental conditions, antigen presented in the absence of a costimulatory signal produced by the B7/CD28 or B7/CTLA4 interaction induces T cell tolerance.

Animal models demonstrate an important role for B7 in inducing antitumor immunity in vivo. Townsend and Allison transfected a K1735 murine melanoma cell line with the murine B7 (21). In comparison to a control vector-transfected K1735 line, the B7 transfected tumor grew poorly in the syngeneic host and was able to protect the host from a subsequent challenge by the parental tumor cell line. Depletion of the CD8+ T cell subset in vivo decreased the immunogenicity of the B7-transfected cell line, and restored the growth rate of the tumor to that observed with the parental or a vector-transfected control tumor line. Depletion of CD4+ T cells did not diminish the immunogenicity of the B7/K1735 tumor cell line, suggesting that B7 costimulation provided by the tumor cells may have bypassed the need for CD4-mediated T cell help.

A separate group of investigators conducted similar experiments using the same melanoma cell line (22). While they reported that B7 transfection did not affect the growth of K1735 in the syngeneic host, tumor size was examined only at one early time point (on day 21). Had the animals been observed for longer periods, the experiments in the former study indicate that most of the B7-transfected tumors would begin to regress as compared to continued growth of the parental cell line. The effect of B7 transfection was more clearly

demonstrated in a K1735 cell line that was further modified by insertion of a strong tumor antigen, the E7 gene product of human papillomavirus. Immunization with the E7+B7+ K1735 cell lines not only protected animals from a subsequent challenge of E7+K1735 tumor, but also delayed growth of a simultaneously placed E7+K1735 tumor in the opposite flank and of an established 4-day old E7+K1735 tumor. Depletion experiments indicated that antitumor effects were mediated by CD8+ T lymphocytes. Immunization with the B7+E7+ transfected cells also induced specific CTL activity against the corresponding B7-E7+ tumor in vivo.

These experiments demonstrate that if a strong antigen is present (ie E7) on the tumor, immunization with the corresponding B7-transfected tumor cell line may result in regression of established tumor. Even if the tumor expresses only weak tumor-associated antigens (ie the parental K1735 cell line), it is still possible to demonstrate that immunization with a B7-transfected tumor cell line will result in an antigen-specific CD8+ T lymphocyte response. In the latter case, additional manipulations such as systemic administration of cytokines, or isolation, in vitro expansion and adoptive transfer of the CTL will probably be necessary to produce regression of advanced tumors.

Description and preclinical studies with three allogeneic melanoma cell lines transfected with B7

Three allogeneic melanoma cell lines were obtained from Dr. Timothy Darrow of Duke University. The cell lines were clonal culture isolates from surgical specimens obtained from three unrelated melanoma patients. The cell lines and haplotypes are as described below:

1. DM-150: HLA-A1, A2, B8, HLA-DR positive
2. DM-13: HLA-A2, A31, B18, (B13, or B40), HLA-DR negative
3. DM-93: HLA-A2, A19 (or Aw33), B8, B49, HLA-DR negative

All three cell lines express moderate levels of LFA-3 (CD58) and ICAM-1 (CD54). ICAM-1 and HLA class I and class II molecules are upregulated by 48-hour in vitro culture with interferon-gamma.

The cDNA for human B7 was isolated by reverse transcriptase/PCR cloning using RNA from the human Burkitt's lymphoma cell line Raji. This PCR product is 970 base pairs in length and encodes the complete protein. The gene was cloned into the Xho I site of vector BCMG-neo, downstream of the cytomegalovirus (CMV) promoter/enhancer, to generate the plasmid BCMGNeo-B7 (Appendix I). Sequences within the BCMG-neo encoding early region genes from the bovine

papillomavirus (BPV 69%) were excised by digesting BCMG-neo with Hind III and Not I. The large DNA fragment resulting from this digestion was gel-purified subcloned into the vector Bluescript SK-. This plasmid (CMV-B7, appendix II) contains the CMV promoter driving expression of the human B7 gene and the SV40 promoter upstream of the neomycin phosphotransferase gene (allowing for selection of transformants by growth in G418). No other eukaryotic genes are expressed, and this plasmid is not capable of episomal replication. The entire 970 bp of the B7 gene contained in the final construct were sequenced and found to match exactly the published sequence for the human B7 gene.

Parental melanoma cell lines DM-150, DM-93, and DM-13 were transfected with this plasmid using the Lipofectin reagent (Gibco/BRL), and G418-resistant colonies were selected utilizing cloning rings to ensure clonality. Twelve clones derived from each parental melanoma line were expanded and analyzed by FACS using the monoclonal antibody BB1 which is specific for B7. One BB1+ clone from each parental cell line was selected for use in this trial; the clones are referred to as DM150/B7-8, DM13/B7-7, and DM93/B7-3. The cell surface phenotype of the clones was assayed by FACS and was identical to the parental cell phenotype with the exception of the expression of B7.

DM150/B7-8, DM13/B7-7, and DM93/B7-3 cell lines have been analyzed for the expression of MAGE and tyrosinase genes. MAGE expression was examined by RT-PCR analyses of RNA extracted from the B7-transfectants. Tyrosinase gene expression was determined by Northern blot analysis. DM-150 transfectants express MAGE-1, 2 and 3 RNA, while DM13 and DM93 express MAGE-2 and 3 but only low levels of MAGE-1. Although tyrosinase was detected in all three cell lines, DM150 expressed only low levels. HLA-A2 TIL cell lines obtained from Dr. Kawakami and Dr. Rosenberg (Surgery Branch, NCI) have demonstrated lytic activity at low effector:target ratios against both parental and B7-transfected melanoma cell lines.

We examined the capacity of the B7-transfectants versus the parental cell lines to stimulate allogeneic peripheral blood lymphocytes (PBL) of a normal donor in a 7-day in vitro culture. PBL were depleted of B cells and macrophages. In 5 separate experiments, parental tumor lines failed to stimulate allogeneic T cells as determined by the lack of increased expression of HLA-DR or CD25 on the T cells. In contrast, B7-transfected lines induced increased expression of CD25 and HLA-DR on both CD4+ and CD8+ allogeneic T cells. The B7-transfectants also induced a 5-10 fold increase in T cell number in comparison to the parental tumor cell lines. Exogenous IL-2 could not rescue T cells cultured with the parental cell line, and had variable stimulatory effects on T cells cultured with the B7-transfectants. Only T

cells cultured with the B7-transfectants developed CTL activity, which was observed against both the B7-transfected and the parental tumor cell line. Furthermore, T cell adhesion to B7-expressing melanoma cell lines was significantly enhanced compared to the parental tumor cell lines and appeared to involve LFA-1/ICAM-1 interactions.

In preparation for the clinical trial, a master cell bank consisting of 10 vials (each vial containing 2-5 x 10^6 cells) for each B7-transfected cell line (DM150/B7-8, DM13/B7-7, and DM93/B7-3) has been established. A working cell bank of 50 vials (each vial containing 2-5 x 10^6 cells) will be established from 1 vial of each master cell bank. One vial of each master cell bank will be tested for mycoplasma, general sterility (fungal and bacterial cultures), and contamination by retroviruses (reverse transcriptase assay). When a patient is identified and has signed an informed consent, one vial of the working cell bank for the designated cell line will be thawed and placed in culture containing fetal bovine serum. The culture medium will also contain penicillin and streptomycin. Cells will be expanded to the appropriate number in sterile flasks. Cell expansion is predicted to require no more than 3 weeks, and will be performed in a laboratory dedicated to expansion of human cells (a LAK lab). After expansion to the desired cell number, the tumor cell line will receive 20,000 rad (lethal irradiation) prior to administration to the patient. A sample of the final product will be examined microscopically prior to administration.

HLA-A2 patients will receive inoculation every 2 weeks x 3, then monthly using one cell line at a time in the following order: DM150/B7-8, DM13/B7-7, and DM93/B7-3. By rotating administration of these lethally irradiated melanoma cell lines to HLA-A2 melanoma patients, we hope to induce a potent in vivo CD8+ CTL response to the common melanoma antigen(s) presented by HLA-A2 while minimizing the response to allogeneic determinants. Since only 1 of our cell lines expresses HLA-A1 and MAGE 1 and 3, patients who are HLA-A1 will receive only the DM150-B7 cell line although the same schedule will be maintained. Dose escalation will proceed separately for the HLA-A1 and HLA-A2 patients. Patients that express both HLA-A1 and HLA-A2 will be treated with the rotation schedule as noted for HLA-A2 patients, since there is preliminary evidence that antigens presented by HLA-A2 are immunodominant. Patients who are HLA-A2 positive will not require a demonstration that their tumor expresses the tyrosinase gene, since Boon et al have shown expression of tyrosinase in 40/40 melanoma tumors and have not detected mutations in the gene sequence. MAGE-1 and 3 are detected in only 60-70% of patients that are HLA-A1. All HLA-A1 patients will be eligible for the study, however, since other as yet undetermined shared melanoma antigens may be presented by HLA-A1.

Where possible, lymphocytes from the draining lymph nodes, tumor, the vaccine injection sites, and peripheral blood will be examined for reactivity against a panel of HLA-A2 melanoma cell lines that do not share the allogeneic determinants of the immunizing cell line. Controls will include malignant non-melanoma HLA-A2 tumor cell lines and autologous EBV-transformed lymphoblastoid cell lines (EBV-LCL).

Immunization with the B7-transfected melanoma cell line may also induce a CD4+ T cell response. Since class II MHC generally presents phagocytosed and not cellular antigens, induction of a CD4+ T cell response would likely occur by macrophage processing of dead tumor cells. It is interesting to note that CD4+ antigen-specific T cell responses have been sufficient to induce tumor regression in some animal models, without the participation of CD8+ cells. It will be of some interest to determine whether our patients develop melanoma-specific CD4 T cell proliferative responses.

We recognize several potential problems. Immune tolerance to the melanoma antigen (perhaps induced by recognition of antigen on tumor cells without costimulatory signals) may be present in vivo and may not be overcome by immunization with the B7-transfected cell lines. Shu et al have shown that immunization with tumor cells plus C. Parvum into the footpad of a non-tumor bearing mouse results in the development of tumor antigen-specific pre-effector CTL in the draining lymph nodes. Full maturation to CTL is accomplished by further culture of pre-effector lymphocytes in vitro with the sensitizing tumor and IL-2. However, in the presence of pre-existing visceral tumor (but not tumor in the subcutaneous tissue), immunization into the footpad fails to elicit the tumor-specific pre-effector T cells in the draining lymph nodes (23). In another series of experiments (conducted at the BRMP), Ochoa and his colleagues have demonstrated that T cells from animals with advanced tumors have defective T cell receptors and receptor signalling (24). Other mechanisms of tolerance have been described including T cell exhaustion by chronic exposure to antigen or the presence of a suppressor CD4+ lymphocyte population. Thus, it may be difficult to induce an antigen-specific CTL response in a patient with advanced melanoma by in vivo immunization, even though the immunizing cell line has been modified to contain costimulatory signals.

Other problems related to the vaccine and the assay for response may be encountered. The effect of the allogeneic determinants present on the B7-transfected cell lines with regard to the immune response to the relevant antigen is unknown. We are also aware that the optimal frequency of immunizations has not been determined. The effect of restimulating activated

T cells with an APC is unknown and may result in apoptosis rather than further expansion of the desired T cell population. Despite these uncertainties, we believe that the existing data warrant initiation of the clinical trial.

II. Objectives

1. To determine the maximum number of B7-transfected allogeneic melanoma cells (the maximum tolerated dose) that can be administered subcutaneously to patients with metastatic melanoma.

2. To characterize the immune response to administration of B7-transfected cells.

3. To characterize the toxicity of the immunizations and to observe any antitumor responses.

III. Cell Culture Methods and Preparation of Cells for Administration

1. The cell culture medium is composed of Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS), G418 (geneticin, Gibco/BRL, 800 mcg/ml), penicillin G sodium (50 units/ml), streptomycin sulfate (50 mg/ml), and sodium pyruvate (0.1 gm/liter). The cells are frozen in cell culture medium containing 20% FBS and 10% DMSO.

2. Frozen cells are stored in liquid nitrogen until needed. An ampule of the appropriate cell line is then removed and transported to the lab in dry ice. The ampule is rapidly thawed in a 37°C water bath, and the cells in freeze medium are gently pipetted into a 15 ml conical tube. Cell culture medium (13 ml) is added slowly to the cells. The tube is spun at 1000 rpm for 5 minutes, and the cell pellet is resuspended in culture medium and placed into a T25 or T75 flask and culture is begun at 37°C in 5% CO_2. Viability of the cell lines are > 90% upon thawing.

3. When the cells become confluent they are transferred into larger culture vessels. Culture medium is removed by aspiration and 4 ml of 0.25 % trypsin (Gibco/BRL) is added per T75 flask. When the cells have detached from the substratum, the cell suspension is passed into a vessel containing fresh culture medium. The cells will ultimately be expanded into Nunc triple flasks and grown until an appropriate number of cells is obtained. One Nunc triple flask supports the growth of greater than 5×10^7 of the human melanoma cell lines used in this protocol.

4. Forty-eight hours prior to harvest an aliquot of the cell supernatant from at least one flask will be sent for bacterial and fungal cultures and contamination by endotoxin. The final harvest of cells for delivery to the patient will proceed as follows: Cells in an appropriate number of triple flasks will be trypsinized and placed in 3-fold excess volume of culture medium at a concentration of 2-10 x 10^6 cells/ml. Cells will then be transported on ice to the gamma-irradiator (Mark-1 irradiator: J.L. Shepard and Associates, San Fernando, Ca.), and 20,000 rads will be delivered over 10.5 minutes. Cells will then be returned to the lab, washed three times in cold PBS, and counted. A gram stain will be performed. Depending on the cohort, cells will be resuspended in an appropriate volume of PBS for injection and aspirated into a capped syringe. Cells will be transported on ice to the clinic. The cells do not stick to the walls of the syringe. The cells will be administered subcutaneously as noted below immediately upon arrival to the clinic.

IV. Eligibility/Exclusions

Eligibility Criteria:

1. Patients with metastatic melanoma. Patients who have stage IV disease that has been completely resected are eligible. The pathology must be reviewed and confirmed by the Surgical Pathology staff of the NCI.
2. Patients must be typed for histocompatibility antigens (HLA-A, B, and C, HLA-DR), and must be positive for HLA-A1 or HLA-A2.
3. Age > 18, male or female
4. Performance status 0 or 1
5. No other treatment for their malignancy within the 4 weeks prior to entry to this study.
6. Life expectancy of 3 months or more.

Exclusions

1. HIV or HBsAg positivity.
2. Women or men of child-bearing age unless a negative pregnancy test is obtained prior to enrollment and adequate methods of contraception are used during the study period.
3. Organ dysfunction:
   a. WBC < 3500, or granulocyte count < 1500
   b. Platelets < 100,000
   c. Hemoglobin < 8.0 mg/dl
   d. PT/PTT > 1.2 x upper limit of normal
   e. Creatinine > 2.0 mg/dl
   f. Bilirubin > 1.5 mg/dl
   g. SGOT/SGPT > 2x upper limit of normal, or alkaline phosphatase > 4x upper limit of normal.
   h. Albumin < 3.0 mg/dl or CT scan evidence that tumor involves greater than 1/3 of the liver parenchyma
   i. Clinical evidence of pulmonary dysfunction (dyspnea at rest or with minimal to moderate exertion).
   j. Significant cardiovascular disease: symptoms of coronary artery disease (includes those controlled with medication), history of MI , congestive heart failure, chronic arrhythmias requiring treatment, peripheral arterial insufficiency.
   k. Evidence or history of brain metastases, or seizure disorders controlled with medication.
4. Active infection requiring antibiotics within one week of starting therapy.
5. Requirement for corticosteroids.
6. Any treatment including radiation therapy for their malignancy within 4 weeks of starting treatment (6 weeks for BRM's).
7. Any medical or psychiatric condition which in the opinion of the treating physician or principal investigator would unacceptably reduce the safety of the proposed treatment, would impair the

delivery of treatment, or would preclude obtaining voluntary informed consent.

8. Patients receiving any other concurrent investigational treatment, or any other concurrent treatment for their cancer.
9. A diagnosis of a second malignancy within the past 5 years except carcinoma in situ of the cervix or basal cell carcinoma.
10. Patients that are allergic to penicillin or streptomycin.

V. Study Design

1. Patients will be stratified by expression of HLA-A1 versus expression of HLA-A2 or HLA-A1/A2. Dose escalation and accrual will proceed independently for these two groups. HLA-A2 patients will be treated every 2 weeks x 3, then monthly using one cell line at a time in the following order: DM150/B7-8, DM13/B7-7, and DM93/B7-3. Patients in the HLA-A1 group will receive treatment in the same schedule but only the DM150/B7-8 cell line will be used. A total of 6 vaccine administrations are allowed. Cohorts of 6 patients each will be accrued consecutively. The number of cells tc be injected per vaccination in each successive cohort will be:

Cohort A1 -1 and A2-1 - 10^7
Cohort A1-2 and A2-2 - 10^8
Cohort A1-3 and A2-3 - 10^9
Cohort A1-4 and A2-4 - `MTD' of a-c with non-transfected parental cell line

At the `MTD' or highest cell dose, a separate cohort of 6 patients (Cohort A1-4 and A2-4) will receive the same dose of the non-transfected parental melanoma cell line(s). This will allow some comparison to be made as to whether transfection of B7 altered the immune response and toxicity to the tumor cells. If ongoing monitoring of these patients reveals evidence that the immune response was suboptimal (the definition for suboptimal response cannot be given until data are available from the cohorts receiving the B7 transfected cells), then one month after the 6th injection (see below), the patients will be eligible to restart the treatment program with B7 transfected cells, presuming they would otherwise meet the eligibility criteria for the protocol and have had stable or slowly progressive disease. Patients with tumor response but a `suboptimal' immune response to non-transfected cells would continue to receive the non-transfected cells as outlined below.

No systemic toxicity is expected with administration of either the transfected or non-transfected cell line. This is based on a very large experience with the administration of allogeneic cell lines in melanoma patients (1,12).

Nevertheless, accrual to a cohort will stop if 2 patients develop grade 3 or grade 4 toxicity (with the exception of local grade 3 toxicity which will not be considered dose-limiting, see below). The next lower dose level will be declared the MTD providing no more than 1/6 has developed a dose-limiting toxicity. Local toxicity will be graded as follows: erythema and induration < 20 mm, grade 1; erythema and induration greater than 20 mm but no ulcer, grade 2; ulcer, or painful regional adenopathy, grade 3; permanent dysfunction related to local toxicity, grade 4.

2. The maximum concentration for mixing cells is 10^8 /ml. At the 10^7 dose level, the cells will be brought up to 1 ml total volume. For the 10^7 and 10^8 dose levels, each dose will be split into two 0.5 ml aliquots and injected 5 centimeters apart (approximately 3 finger breadths) into the same extremity. At the 10^9 dose level, each dose will require 10 separate injections of 1 ml placed 3 cm apart into the same extremity. All doses will be injected subcutaneously in an extremity with intact draining lymph nodes. Injections will be given every 2 weeks x 3, then monthly x 3 (see V.1). Sites will be rotated as follows: right leg, left arm, right arm, left leg, depending on the presence of draining lymph nodes. Each injection site will be marked.

3. There will be no dose reductions for toxicity. If grade 3/4 toxicity is observed (other than local grade 3 toxicity), the patient will be removed from the study.

4. A surgical biopsy of one of the vaccination sites will be performed in consenting patients 2 weeks after the first and fourth injections. Sampling (removal) of a superficial lymph node (if enlarged secondary to vaccination) is planned 2 weeks after the first and fourth injections.

5. Tumor measurements are obtained just prior to the 4th injection, then every other month. Treatment should continue as long as patients are found to have stable disease or evidence of an objective response. A maximum of 6 vaccine injections is allowed. Patients with slowly progressive disease (as determined by the treating physician) on their first evaluation (prior to the fourth injection) may continue treatment. Patients with rapidly progressive disease noted at any time during the study period should be taken off study. Every effort will be made to put patients with progressive disease on a new BRMP protocol containing systemic administration of
IL-2.

6. Supportive Care = Concurrent treatment with steroids, other anti-neoplastic therapy, colony-stimulating factors or other investigational drugs is

prohibited. All ancillary medications used for symptomatic therapy should be accurately recorded on the case report forms.

VI. Study Endpoints and Monitoring - (Appendix III)

1. The major laboratory endpoint of this study is to determine whether the B7-transfected melanoma cell lines induce cell-mediated T cell responses against the antigen(s) presented by HLA-A2 and HLA-A1. Lymphocytes will be obtained from 4 possible sites: the vaccine injection site, the draining lymph nodes, PBL, or an accessible tumor. If possible, lymphocytes will be tested directly against the targets, or will first be co-cultured with low-dose IL-2 and with the immunizing cell line or matched allogeneic HLA-A2 or HLA-A1 melanoma cell lines that do not share any of the allo determinants of the immunizing transfected cell line. Co-cultures with B7 transfectants of the melanoma cell lines will also be considered.

The targets will be the immunizing cell lines (wild type and B7 transfected), several HLA-A2 or HLA-A1 melanoma cell lines not sharing the allogeneic determinants of the immunizing cell line, HLA-A2 or HLA-A1 non-melanoma tumor cell lines, and perhaps autologous EBV transformed -lymphoblastoid cell lines (EBV-LCL). Measures of reactivity will include cytotoxicity in 4 or 18 hour chromium release assays or cytokine production (interferon-gamma and TNF). Frequency of CTL will be determined by limiting dilution assays (25). In addition, proliferation assays will be performed against the panel of targets, in some cases after up-regulation of class II antigens with interferon-gamma.

If antigen-specific CTL are isolated, a variety of experiments will be performed to learn how to optimally expand the cells in vitro. Although not a primary endpoint of the current study, these laboratory efforts will be designed to generate data for the next generation of clinical protocols.

PBL samples will be obtained x 3 prior to treatment, then at 7, 14 (prior to the second injection), 21, 28 (prior to the third injection), 42, 56, 84, 112, 140 and 168 days after the first injection, then every scheduled visit in patients who remain on study with stable or responding disease. LN and biopsy sample times are described above. Tumor biopsies when available will be obtained 2-4 weeks after the 4th or subsequent injections.

Skin test reactivity to the non-transfected cell line DM-150 and will be tested at the time of administration of the 4th vaccination with the B7-transfected cell line. 10^7 lethally irradiated cells will be used and will be injected in the opposite extremity. The area of induration will be measured

using standard techniques at 24, 48, and 72 hours. A punch biopsy of the site will be considered 2 weeks after placement of the skin test.

2. Serologic responses to the allogeneic determinants and to a panel of HLA-A2 and HLA-A1 melanoma cell lines not sharing the allogeneic determinants will be measured. Samples will be obtained x 3 baseline, then at 7, 14 (prior to the second injection), 21, 28 (prior to the third injection), 42, 56, 84, 112, 140 and 168 days after the first injection, then every scheduled visit in patients who remain on study with stable or responding disease.

3. Safety monitoring - Pre-study:

Complete history and physical
PBL for HLA-A, B, C, and HLA-DR typing
Determination of penicillin/streptomycin allergy by history
CBC
CHEM 20
PT/PTT
UA
CxR
EKG
HIV/HBsAg
CT scans of head, chest and abdomen
Other scans as necessary to follow disease
IP x 3 to determine immune competence (ie proliferative response to mitogens and recall antigens, T cell receptor abnormalities, PBL phenotyping)

All prestudy tests should be performed within 4 weeks of receiving the first vaccination. Tests required for tumor measurements should be completed no more than 4 weeks prior to the first dose. On day 0 (day of first vaccination) a limited interval history and physical and CBC/CHEM 20 should be obtained.

4. Safety Monitoring - During Study

a. On the day of vaccine administration, patients will be observed for 4 hours after the injection. Vital signs will be obtained hourly during observation. After the first and second injection, the patients will be examined at 24 and 48 hours with special emphasis on the injection sites and regional lymph nodes. An H/P, CBC, chem 20, and U/A will be done weekly for the first four weeks, then every other week x 2, then monthly. CxR is obtained at one month.

b. See above for schedule of tumor measurements.

5. Safety Monitoring - Completion of Treatment

Patients will be seen 4 and 8 weeks following the last vaccination, then every other month x 3, then every three months x 4, then every 6 months until progressive disease. An H/P, CBC, chem 20, and U/A will be done at each visit. Tumor measurements are scheduled 8 weeks following the last vaccination, and subsequently every scheduled visit as above. Blood will be drawn for serologic and CTL responses (see section VI) at the 4 and 8 week visit after the last vaccination, then at every scheduled visit.

VII. Criteria for Response

Complete remission (CR) - disappearance of all evident tumor (based on symptomatic, clinical, and radiographic resolution) and return of tumor-related abnormal tests to normal levels for a 28 day period. Abnormalities remaining which are believed not to represent tumor would require biopsy demonstrating the absence of malignant cells.

Partial remission (PR) - a 50% or greater decrease in the sum of the cross sectional areas of all measured lesions, in the absence of progression of any lesion or appearance of any new lesions for a 28 day period.

Stable disease (SD) - any change in measurable disease too small to meet the requirements of partial remission (50%) or progression (>25%) without new lesions.

Progression (PD) - any of the following criteria: Development of any new lesion; increase of any previous single lesion by >50% in bidimensional diameter; new or progressive ascites, pleural effusions, jaundice, or decline in neurologic status not explained by other factors. Significant clinical deterioration related to malignant disease of two or more performance levels will be considered progressive disease.

VIII. Criteria for Discontinuation of Treatment

1. Progressive disease (see section V.5).
2. Intercurrent illness which prevents further administration of treatment.
3. Unacceptable toxicity.
4. Decision of the patient to withdraw from the study.

5. General or specific changes in the patient's condition which render the patient unacceptable for further treatment in the judgement of the investigator.

IX. Reporting of Adverse Drug Reactions

Adverse drug reactions will be reported to the DCT/NCI using the DCT/NCI Adverse Reaction Form for Investigational Agents. Toxicity will be graded according to the NCI Common Toxicity Criteria (Appendix 4). The telephone number and FAX for reporting ADR's is available 24 hours per day (recorder after working hours) as follows: 301-230-2330 and fax 301-230-0159. All written reports should be mailed to:

Investigational Drug Branch
P.O. Box 30012
Bethesda, Maryland 20824

Adverse drug reactions will be reported as follows. Report by telephone to IDB within 24 hours (301-230-2330, fax 230-0159):

a. All life-threatening events (grade 4) which may be due to drug administration.
b. All fatal events.
c. The first occurrence of any previously unknown clinical event (regardless of grade).

Written report to follow within 10 working days.

X. Statistical Section

This is a feasibility study. The major clinical endpoint is establishment of a safe dose of B7 transfected melanoma cell lines that can be administered repeatedly.

The major laboratory endpoint is to determine whether the transfected and non-transfected cell line induces a specific T cell response to the melanoma antigen(s) presented by HLA-A1 and HLA-A2. Comparisons will be made within patients to their baseline response, and between groups receiving transfected versus non-transfected cells. A 1-log increase in any measured parameter is considered biologically meaningful.

XI. Collection of Data and Data Submission

1. Data will be submitted to CTMS at least once every two weeks from each participating institution.
2. The NCI/DCT case report form or ACES will be used to report to CTMS.

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XIII. Consent Form - CONSENT TO PARTICIPATE IN A CLINICAL STUDY

Institution: Clinical Research Branch, Biological Response Modifiers Program, NCI
Study Number: T93-XXXX, BRMP 9401
Principal Investigator: Mario Sznol, M.D.
Study Title: A Phase I trial of B7-transfected lethally irradiated allogeneic melanoma cell lines to induce cell-mediated immunity against tumor-associated antigen presented by HLA-A2 or HLA-A1 in patients with stage IV melanoma

Introduction

We invite you to take part in an experimental, research study at the BRMP. It is important that you read and understand several general principles that apply to all who take part in our studies:

(a) taking part in the study is entirely voluntary;

(b) personal benefit may not result from taking part in the study, but knowledge may be gained that will benefit others;

(c) you may withdraw from the study at any time, or refuse to participate, without penalty or loss of any benefits to which you are otherwise entitled.

Your doctors have told you that standard therapies are no longer controlling your cancer. For this reason we are asking you to participate in this investigational study. The treatment that you are being offered may be helpful to control your disease. However, it is investigational and therefore it is not possible to determine the likelihood of benefit to you at this time.

Treatment

Scientists have recently discovered that the tumors from some patients with melanoma contain a protein (called an antigen) that can be recognized by the immune system. This means that blood cells called lymphocytes could attach to the tumor cells and recognize that the tumor cells are not normal and don't belong in the body. Once the lymphocyte determines that the tumor cells are not normal, it would kill them or call other blood cells to the site that would help eliminate the tumor cells. Although the potential for this immune response exists in some patients with advanced melanoma, for reasons that we still do not understand, the immune system has somehow been paralyzed and

cannot recognize or attack the tumor.

We have grown tumor cell lines in the laboratory that we believe contains the same tumor antigen that your tumor has. We have taken a gene which codes for a molecule called B7 and placed it in the melanoma tumor cell lines we have in the laboratory. We believe that the tumor cell lines containing the B7 will be more able to activate your lymphocytes to recognize tumor antigens and that this recognition could cause them to attack your tumor.

In order to administer treatment, we must first determine if your body cell type matches the tumor cell lines we have in the laboratory. We do this by determining the expression of specific proteins on the surface of your lymphocytes that we obtain prior to the study. The two body cell types that match the tumor cell lines we have in the laboratory are HLA-A1 and HLA-A2. If your lymphocytes do not have the markers HLA-A1 or HLA-A2, you cannot receive treatment on this study because your tumor and our tumor cell lines would not be matched, and we would attempt to find another treatment for you.

For treatment, we plan to inject a large number of cells (which are first killed by radiation before you receive them) from the tumor cell lines containing B7 under your skin. The cells are irradiated to kill them so there is little chance that a tumor might grow where we inject the cells. The killed tumor cells are injected every two weeks for 3 doses, then once a month for 3 doses. We rotate the sites of injection, usually starting in the leg, then the arm, then the opposite arm, then the opposite leg, and so on. If you are HLA-A1 as described above, you receive the same dose of the same cell line each time. If you are HLA-A2, we alternate treatments with one of three cell lines. In either group, you can receive a maximum of 6 treatments.

An important part of this trial is to determine how many cells we need to give and what side effects they will have. We do not anticipate any dangerous side effects. Therefore, we will treat groups of patients with each group receiving a larger number of killed cells. The purpose of the study is to determine how many killed cells we can give safely and whether you develop an immune response to the cells as we predict from the laboratory studies. If you are in the first or second group of patients, you receive 2 injections (about 2 inches apart) each time you are scheduled for treatment. If you are in the third group, you receive 10 injections each time. Each injection will take only a few minutes.

We do not know if the B7 actually improves the immune response to the cells. Therefore, some of you may receive the same killed tumor cell line but not

containing B7. If you receive cells not containing B7 and you don't have a good response after receiving all 6 planned injections, we may try to give you the cells containing B7 in the same way as long as you are still in good enough condition to continue treatment on this protocol. You will be told prior to entering the study whether you would receive the cells with B7 or the cells without B7.

As long as your tumor is stable or shrinking, you may continue on the therapy up to a maximum of 6 treatments. Before, during and after treatment you will have x-rays, blood samples taken, and other lab tests performed to monitor your therapy. We will ask if we can remove a small area around the site of an injection 2 weeks after the first and fourth injections for investigational purposes. This will involve minor surgery that is performed with local anesthesia. We will also remove for investigational purposes, with your permission, any swollen surface lymph nodes or surface tumors at similar times. The risks of these biopsies include bleeding, infection, or poor healing leading to a chronic open wound or ulcer. However, the chances of complications occurring from a local surgical procedure are low. If you refuse to have these minor surgeries you may still participate in the study.

Side Effects

The extent and severity of side effects cannot be totally predicted. It is possible you may develop redness, swelling, or an ulcer at the place where the injection has been given. The lymph nodes in your groin or arm may become tender and swollen. Other side effects may occur.

Alternative Treatments

Your doctors are very willing to discuss the benefits and side effects of alternative treatments including the option of treatment of your symptoms only with no further cancer therapy. Other investigational protocols with chemotherapy, hormones, radiation therapy, or novel anti-cancer agents may be available for your disease. Please ask questions you may have and take as much time as you need to make your decision.

Confidentiality

When results of a study such as this are reported in medical journals or at meetings, identification of those taking part is withheld. Medical records of patients are maintained in strictest confidence according to current legal requirements. In the past, studies such as this one that involved genetic

modification of a cell received a great deal of news coverage. Although the studies are more common today, they may still receive some attention by the news media. Under no circumstances will your identity or your participation in this study be revealed. However, your medical records are made available for review to the National Cancer Institute or the FDA, as required by the Food and Drug Administration (FDA) or other authorized users only under the guidelines established by the Federal Privacy Act.

Compensation

You will not be paid for taking part in this research study.

Problems or Questions

The physicians involved in your care have been available to answer your questions about this treatment program and will continue to be available in the future. A copy of this consent is kept on file and a copy will be given to you. You may contact the principal investigator Dr. Mario Sznol or other staff members at 301-846-1520 with questions about this study, about your rights as a research subject, or about any injury you feel is related to this study. If there are any questions as to your rights as a patient, you may contact the patient representative, Ms. Jane Snider, at (301) 698-3537.

Research Related Inquiries

The BRMP will provide short-term medical care for any physical injury resulting from your participation in research here. However, the BRMP will not provide long-term medical care or financial compensation for such injuries, except as may be provided through whatever remedies are normally available under law.

Additional Costs

By your participation in this study, you may incur additional costs not covered by the BRMP, the hospital or by your insurance company. You will be responsible for these payments.

Voluntary Participation

Participation in this study is entirely voluntary. You may choose not to participate or you may withdraw at any time. We will continue to offer you the best care we can, whatever you decide. You may be interested in knowing

the circumstances under which the research would be terminated. Research involving drug therapy may be terminated by your physician should you not receive benefit or should life-threatening side effects occur. Should new information become available regarding a superior treatment for your type of disease, you will be informed and you should discuss further therapy with your doctor at that time.

AIDS Antibody Test

As part of your participation in this protocol, your blood will be tested for antibodies to the human immunodeficiency virus (HIV), the virus that causes AIDS. If you are found to have these antibodies, or if you have been diagnosed as having AIDS you should be aware of the following policy: 1) your doctor will notify you promptly of the result, 2) your doctor will offer you, your spouse, and current and/or ongoing sexual partner(s) whom you identify, information on the meaning of the test and how to prevent the spread of this infection, 3) because HIV can be transmitted in several ways, it is important that you inform these partners that any or all may have been exposed to the HIV virus, and encourage them to be tested. If you request, we will assist you in notifying your current and/or ongoing partner(s), and arrange counseling, 4) in the event that you are unwilling or unable to notify any or all of these partners, we are responsible for attempting to assure that they have been made aware of their possible exposure to HIV. All reasonable attempts will be made to protect your identity. (For example, the partner(s) will be notified that they have been exposed to HIV without naming the individual who exposed them). Some of these notification and counseling procedures may be carried out through arrangements with, or referral to, local public health departments.

In addition, if you are found to have this antibody in your bloodstream, you will not be eligible for entry onto this study. The treatments that are administered on this study that you would otherwise enter may alter the immune system in such a way that the treatment might worsen the viral infection.

Authorization

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. My signature indicates that I consent to take part in this study and that I have received a copy of the consent form.

_____	_____
Signature of Patient	Date
_____	_____
Signature of Witness	Date
_____	_____
Signature of Physician	Date

CURRICULUM VITAE

Name: Mario Sznol, M.D.

Date and Place of Birth: September 18, 1957; Havana, Cuba

Citizenship: United States

Marital Status: Married, 1984; three children

Education:

1975 - Graduated Bellaire High School, Houston, Texas
1979 - B.A., Rice University, Houston, Texas
1982 - M.D., Baylor College of Medicine, Houston, Texas

Board Certification:

1985 - Diplomate, American Board of Internal Medicine
1987 - Diplomate, Subspecialty, Medical Oncology

Brief Chronology of Employment:

1982-1985 -	Internal Medicine Residency, Baylor College of Medicine Affiliated Hospitals
1985-1987 -	Fellowship, Medical Oncology, Department of Neoplastic Diseases, Mt. Sinai School of Medicine
1987-Date -	Senior Investigator, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute;

Societies:

American College of Physicians, 1991-present
American Association for Cancer Research (AACR), 1991-present
American Society of Clinical Oncology, 1989-present

Editorial Advisory Board:

Journal of the National Cancer Institute, 1992-

Honors and Other Special Scientific Recognition:

1975, National Honor Society
1975, National Merit Scholarship
1975, Cuban Association of Professionals and Students Scholarship
1979, Will Rice College Fellow
1979, Magna Cum Laude
1979, Phi Beta Kappa
1980, Basic Science Honors
1982, Merck Manual Award

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Date of RAC Approval: 12/3/1993
Date of NIH Approval: 4/19/1994

Scientific Abstract

The goal of immunotherapy is to stimulate the immune system by modification of tumor cells or expansion of lymphocytes which respond specifically to tumor antigens. In this study, we will apply techniques of direct gene transfer to enhance immune response against tumors *in vivo*. Patients with advanced cancer who have failed all effective therapy will be treated by injection of a DNA/lipid complex directly within the tumor. DNA will be used which encodes a heterodimeric cell surface protein recognized in the transplantation response. These genes include the HLA-B7 histocompatibility antigen and β-2 microglobulin gene in a non-viral plasmid eukaryotic expression vector. For this vector, a safe and effective dose to introduce this recombinant gene in HLA-B7 negative patients will be established. HLA-B7 expression will be confirmed *in vivo*, and the immune response stimulated by the expression of this antigen will be characterized. We will also determine whether this treatment facilitates tumor regression. This study employs the same study drug as Dr. Gary Nabel of the University of Michigan, previously proposed gene therapy protocol, but expands the study to two other clinical sites, the Mayo Clinic and the Arizona Cancer Center. These studies will facilitate the development of other approaches, using different recombinant genes or in combination with cytokines or adoptive T-cell therapy, to augment tumor immunity, and allow for greater potential efficacy. This method will also establish the safety on this non-viral approach to gene therapy, which could potentially be extended to treat a variety of other human diseases.

Non-Technical Abstract

Many types of cancer cannot be cured by traditional medical treatments, including drugs, surgery, or radiation. In this study, an experimental treatment will be offered that may help to fight this disease. We will attempt to induce tumor regression by the introduction of genetic material that directs the synthesis of proteins which stimulate the immune system. The genetic material, DNA, will be introduced directly into the tumor by mixing it with fatty substances, or lipids, and this mixture will be injected into the tumor. The DNA will be taken into cells and cause them to produce proteins that stimulates tissue rejection. These proteins, called histocompatibility proteins, cause cells which contain it to be recognized as foreign by the immune system. The goal of the treatment is to stimulate the immune system to attack and kill the tumor. In this study, we will attempt to determine the optimal safe and effective dose to administer the DNA/lipid complex. Increasing amounts of this complex will be used in different patient populations. If no side effects are observed, repeated treatments will be instituted. The expression and nature of the immune response will also be characterized. This treatment may provide a therapeutic effect in cancer and could be applied to the treatment of other diseases.

Phase I Study of Immunotherapy of Advanced Colorectal Carcinoma by Direct Gene Transfer Into Hepatic Metastases

Protocol

Principal Investigator:	Joseph Rubin, M.D.
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Date:	October 5, 1993

Schema

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Schema

Prior to discussing protocol entry with the patient, call the Randomization Center to insure that a place on the protocol is open to the patient.

Escalating doses will be evaluated. Groups of patients will receive either escalating doses of an intralesional injection of the test agent into one metastatic lesion in the liver in a single session (schedule A) or repeated injections at the same dose (schedule B).

Schedule A

Group	Number of Patients	µg of DNA
A-1	3	10µg day 1 only
A-2	3	50µg day 1 only
A-3	3	250µg day 1 only

Schedule B
(Will be initiated after safety of Schedule A at 50µg has been established)

Group	Number of Patients	µg of DNA
B-1	3	10µg day 1 and 15
B-2	3	10µg day 1, 15 and 30

Schedule A

⊇ PR → may be retreated once

REG Rx (day 1) → 4 wks Eval

< no Rx → Obs

Schedule B

⊇ PR → cycle of 2 injections 15 days apart may be repeated once.

B_1 REG Rx (day 1) → Rx (day 15) 4 wks Eval

< PR → no Rx → Obs

⊇ PR → cycle of 3 injections each 15 days apart may be repeated once.

B_2 REG Rx (day 1) → Rx (day 15) 2 wks Rx → Eval (day 30)

⊇PR → no Rx → Obs

1.0 Background

1.1 Overview

Cancer is a disease in which certain cells grow uncontrolled by the body's normal self-regulatory mechanisms. Traditional chemotherapy seeks to control cancer by killing rapidly dividing cells or by preventing cells from entering cell cycle and dividing. However, a number of non-malignant cells in the body such as bone marrow cells and intestinal epithelium cells, are also rapidly dividing and hence are highly susceptible to the toxicity of chemotherapy. Doses sufficient to induce remission in the cancer cannot be administered without life-threatening side effects in 5-10% of the patients and the overall mortality from chemotherapy is 0.5%. A therapeutic approach that selectively kills tumor cells with high efficacy would theoretically be far superior to currently available therapies.

The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells by modifying the tumor cells or modifying the host response by such mechanisms as expanding the lymphocytes that respond specifically to the antigens on the tumor cells. Immunotherapy has shown promise as an approach to the treatment of malignancy. Indeed, cancers such as melanoma, renal cell carcinoma and colon adenocarcinoma are responsive to modulation of immune function, because the immune system can be induced to recognize tumor associated and tumor specific antigens in these cells.

Over the last several decades, there have been many attempts to identify tumor-specific antigens that might be the targets for cytotoxic antibodies or cell-mediated immunity. There have been numerous attempts to develop vaccines and monoclonal antibodies directed at one or more preferentially expressed cell surface antigens in a variety of cancers. Overall, tumor vaccines using intact cells or extracts plus adjuvants have given about a 10-20% response rate. Other approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as Bacillus Calmette-Guerin (BCG), cytokines, and/or adoptive transfer of cytotoxic T cells, which have shown promise in animal models (1-6) and in man (7-10). More recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy.

Nabel and colleagues at the University of Michigan are investigating a novel molecular genetic intervention for human malignancy that enhances the immune response to tumors by in vivo gene transfer. This immunotherapeutic approach based on animal model work (11, 12) uses a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, HLA-B7, introduced into human tumors in vivo by DNA/lipid complex transfection. The direct intratumoral injection approach is used. Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the transfected cells as well as previously unrecognized antigens present in unmodified tumor cells. The introduction of an allogeneic MHC

gene directly into tumors in vivo has induced partial tumor regressions, as well as specific cytotoxic T cell responses to other antigens.

In a preliminary trial in humans with malignant melanoma Nabel treated 5 patients with malignant melanoma. Three patients received 3 treatments, totaling 0.87 μg of DNA intratumorally, and 3 patients (2 additional patients plus one of the original 3 patients) received cumulative dose of 2.58 μg of DNA via three treatments. No toxicity resulted from this form of treatment and there was no formation of anti DNA antibody or autoantibody. There was no plasmid DNA detectable in the blood by PCR following gene transfer (tested on days 3-7 post transfection at ~2 pg/ml sensitivity).

Evidence of gene transfer was found on biopsy of the injected tumor. The biopsy samples were analyzed for plasmid DNA, mRNA coding for HLA-B7 and the expression of HLA-B7 protein. In 4 of the 5 patients, plasmid DNA and HLA-B7 mRNA were detected within the treated nodules by PCR. HLA-B7 expression was confirmed in all treated nodules by immunohistochemical staining with a monoclonal antibody to the gene product. Two patients, where cell lines were established from the tumor, showed an immune response by lysing autologous tumor cells. One of the 5 patients had a partial remission which involved cutaneous and visceral disease. (Nabel, et al., PNAS, in press).

These data suggest that tumor cells modified with the HLA-B7 gene not only stimulate CTLs and potentially other immune system cells to recognize tumors expressing HLA-B7, but they may also provide a stimulus to immune cells to eliminate tumor cells at other sites which express tumor associated antigens in association with the patient's own HLA antigens.

Several improvements that may increase the convenience, safety and efficacy of the procedure have been introduced since the original Nabel studies including:

- o an improved cationic lipid formulation, DMRIE/DOPE*;
- o DNA plasmid construction to optimize expression

The efficacy of transfection was improved for the following reasons. Briefly, a new formulation of cationic lipids has been described recently by Dr. Phillip Felgner (Vical) in which a different cationic lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), is utilized with dioleoyl phosphatidylethanolamine (DOPE). This has two properties which make it more suitable for these studies. First, it shows up to 10-fold improved transfection efficiency in vitro compared to the formulation previously used by Nabel. More importantly, this formulation does not aggregate at high concentrations. This characteristic thus allows higher absolute concentrations of DNA and lipid complex to be introduced into experimental animals

* DMRIE: 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium DOPE: dioleoyl phosphatidylethanolamine bromide.

without toxicity. Because of these properties, it now becomes possible to introduce 100-1000 times more DNA which could allow the study of an expanded dose response gene expression in vivo.

The vector improvements are divided into two categories for this proposal. In the first case expression of the HLA-B7 vector has been improved by the addition of a consensus translation initiation sequence and removal of an intron. In addition, the inclusion of the β-2 microglobulin gene, with which class I MHC genes normally associate, allows synthesis of the complete histocompatibility molecule, which is composed of these two chains. Ordinarily, these two gene products are co-transported to the cell surface. This is important because some human melanoma cells do not express endogenous β-2 microglobulin, thus limiting their ability to stably express class I on the cell surface. It has been found that the inclusion of the β-2 microglobulin gene on the same plasmid allows for the expression in these otherwise resistant cells and improve expression in other cells, thus overcoming a potential mechanism of resistance. These modifications have been incorporated in the study drug to be used in this submission. The study drug is identical to the study drug fully characterized in Dr. Nabel's RAC submission of June 7, 1993, which was unanimously allowed. This study will investigate the administration of the study drug in malignant melanoma.

1.2 Background

1.2.1 Direct Gene Transfer and Modulation of the Immune System

The utilization of catheter-based gene delivery in vivo provided a model system for the introduction of recombinant gene-specific sites in vivo. Early studies focused on the demonstration that specific reporter genes could be expressed in vivo (13,14). Subsequent studies were designed to determine whether specific biologic responses could be induced at sites of recombinant gene transfer. To address this question, a highly immunogenic molecule, a foreign major histocompatibility complex (MHC), was used to elicit an immune response in the iliofemoral artery using a porcine model. The human HLA-B7 gene was introduced using direct gene transfer with a retroviral vector or DNA lipid complex (12). With either delivery system, expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall. More importantly, the expression of this foreign histocompatibility antigen induced an immunologic response at the sites of genetic modification. This response included a granulomatous mononuclear cell infiltrate beginning 10 days after introduction of the recombinant gene. This response resolved by 75 days after gene transfer; however, a specific cytolytic T cell response against the HLA-B7 molecule was persistent. This study demonstrated that a specific immunologic response could be induced by the introduction of a foreign recombinant gene at a specific site in vivo. Moreover, this provided one of the first indications that direct gene transfer of specific recombinant genes could elicit an immune response to the product of that gene in vivo (12).

These studies suggested that the introduction of the appropriate recombinant genes could be used to stimulate the immune system to recognize its product in vivo. In addition, this approach provided a general method for the induction of a specific site in vivo. To determine whether direct gene transfer might be appropriate for the treatment of disease, a murine model of malignancy was developed. Direct gene transfer of an allogeneic histocompatibility complex gene into a murine tumor elicits an immune response not only to the foreign MHC gene but also to previously unrecognized tumor-associated antigens. These immune responses are T cell-dependent, and these tumor-associated proteins are recognized within the context of the self major histocompatibility complex. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (11). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors have potentially therapeutic effects that may be appropriate for the treatment of malignancy.

1.2.2 Immunotherapy of Malignancy

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular and humoral immune effectors. Cellular mediators of anti-tumor activity include MHC-restricted cytotoxic T cells, natural killer (NK) cells (15,16) and lymphokine-activated killer (LAK) cells (17). Cytolytic T cells which infiltrate tumors have been isolated and characterized (18). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (3,19). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (20,21), or by activation induced by substances such as BCG (22).

Cytokines can also participate in the anti-tumor response, either by a direct action on cell growth or by activating cellular immunity. The cytostatic effects of tumor necrosis factor-α (TNF-α) (23) and lymphotoxin (24) can result in neoplastic cell death. Interferon-γ (IFN-γ) markedly increases class I MHC cell surface expression (25,26) and synergizes with TNF-α in producing this effect (27). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (28), and interleukin-2 (IL-2) activates Leu-19+ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (17,29,30). The LAK cells lyse tumor cells without preimmunization or MHC restriction (31). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor specific killer cells (32).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanism to escape host defenses, perhaps through evolution of successively less immunogenic clones (33). Deficient expression of class I MHC molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC

antigen completely or show decreased expression (34-38). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (39-43). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor (40,41). The absolute level of class I MHC expression however, is not the only factor which influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression did not display altered tumorigenicity compared to the parent line (44).

The immune response to tumor cells can be stimulated by systemic administration of IL-2 (45), or IL-2 with LAK cells (46,47). Clinical trials using tumor infiltrating lymphocytes are also in progress (48). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of tumor cells transfected with an IL-2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte response which protected against subsequent rechallenge with the parental tumor cell line (49). Expression of Il-4 by plasmacytoma or mammary adenocarcinoma cells induced a potent anti-tumor effect mediated by infiltration of eosinophils and macrophages (50). These studies demonstrate that cytokines, expressed at high local concentrations, are effective anti-tumor agents.

Nabel and co-workers have previously proposed an alternative approach to stimulate an anti-tumor response, through the introduction of an allogeneic class I MHC gene into established human tumors. The antigenicity of tumor cells has been altered previously by the expression of viral antigens through infection of tumor cells (51-55), or expression of allogeneic antigens introduced by somatic cell hybridization (56,57). Allogeneic class I MHC genes have been introduced into tumor cells by transfection and subsequent selection in vitro. These experiments have produced some conflicting results. In one case, transfection of an allogeneic class I MHC gene ($H\text{-}2L^d$) into an $H\text{-}2^b$ tumor resulted in immunologic rejection of the transduced cells and also produced transplantation resistance against the parent tumor cells (58). In another instance, transfection of $H\text{-}2^b$ melanoma cells with the $H\text{-}2D^d$ gene did not lead to rejection (59), however increased differential expression of H-2D products relative to H-2K may have affected the metastatic potential and immunogenicity of tumor cells (60). The effects of allogeneic H-2K gene expression in tumor cells was examined in another study (61). Several subclones which were selected in vitro and expressed an allogeneic gene were rejected in mice syngeneic for the parental tumor line, however, other subclones did not differ from the parental, untransduced line in generating tumors. This finding suggests that clone-to-clone variation in in vivo growth and tumorigenic capacity may result in other modifications of cells caused by transfection or the subcloning procedure, which affects their tumorgenicity. These types of clonal differences would likely be minimized by transducing a population of cells directly in vivo.

Because the H-2K class I MHC antigen is strongly expressed on most tissues and can mediate an allogeneic rejection response, we chose it in our animal model studies designed to enhance the immunogenicity of tumors in vivo. These studies extended previous efforts to modify tumor cells by developing a system for the direct introduction of genes into tumors by in vivo infection using retroviral vectors or by DNA lipid complex mediated transfection. This technology can also be used to deliver specific recombinant cytokines into the tumor microcirculation and to understand the immunologic basis for tumor rejection in vivo.

The Department of Diagnostic Radiology, May clinic-Rochester, has extensive experience in CT and sonographically- guided needle biopsy (see reviews by Charboneau et al., 1989, and Charboneau, 1991). Sonographically– guided biopsy of metastatic lesions in the liver can be carried out with a high degree of accuracy and safety. Overall accuracy was 91% in the biopsy of 126 consecutive masses of various histologic types 3cm or less in diameter in various anatomic locations. The accuracy increases to 98% for masses 2-3 cm in diameter, the range to which we will restrict delivery of DNA/liposome complex. Current techniques are not limited to this caliber (21-22) gauge needles). It is almost always possible to obtain a core rather than an aspirate of tissue by using a 16-19 gauge needle, needles with cutting ends, and improved biopsy sampling technology. In a study from the Mayo Clinic of 1,000 consecutive CT-guided biopsies, the rate of complication from the use of an 18-gauge biopsy needle was 0.3%, the same as the rate of complication from the use of a 21-gauge needle (Welch et al., 1989).

This capability allows in one sitting biopsy of the intended target for gene transfer, documentation of metastatic cancer in the lesion, and delivery of the DNA/liposomal mixture to the targeted nodule(s). Virtually any region of the liver can be biopsied with safety. For purposes of this proposal, noduled 2-4 cm in size that are easily accessible will be selected to further ensure successful target injection and to reduce the possibility of morbidity.

One advantageous feature of sonographically-guided delivery of DNA/liposomal complex is that the distribution of the fluid within the injected area can be visualized and documented on videotape. Apparently, the release of small bubbles from dissolved air in fluids at room temperature injected into tissue causes the area perfused to become hyper echoic. This allows the radiologist to observe the regions of the nodule being injected.

State-of-the-art facilities exist at Mayo for carrying out sonographically-directed biopsy and delivery of materials at deep sites in the body. Dr. Charboneau and his colleagues have now used 95% ethanol as an ablative agent in over 20 patients with hepatic tumors who were not candidates for other surgical or medical therapy. This has involved injecting solitary or, occasionally, up to three intrahepatic masses 5 cm or less in diameter. In most instances, 2-8 ml of ethanol is injected into multiple regions of

an individual mass. the procedure is done with local anesthesia in an outpatient setting, and has been repeated 2 to 5 times over several weeks, provided regression is induced and appears to be continuing. For the in vivo gene transfer study, patients will be admitted to the Mayo General Clinical Research Center (GCRC) in Saint Mary's Hospital. There is an ultrasound suite with its own holding area two floors removed from the GCRC. Invasive procedures in the ultrasound suite are carried out under conditions in which a general surgeon is immediately available in the extremely rare instance of the acute complication of bleeding. An operating room is always immediately available for such circumstances. Following the procedure, patients are kept in the diagnostic radiology area for two hours to assure stability before being transferred, in this instance, back to the GCRC. The suite contains CT scanning equipment, a procedure than can be used to visualize local bleeding.

The chemotherapy, pharmacy, and pharmacology shared resources of the Mayo Comprehensive Cancer Center are available and will be sued to support this project. Darryl Grendahl., B.S. Pharm., is in charge of the Mayo Chemotherapy Shared Resource and is a member of the Department of Pharmacy, Rochester Methodist Hospital. He will assure the storage, preparation, and delivery of the final DNA/liposomal product to the ultrasound suite when needed.

The design of this trial is similar to standard phase I clinical trials. HLA-B7 negative patients with colorectal cancer and hepatic metastases, two of which are clearly measurable by CT scan and one of which is appropriate for sonographically-guided biopsy and injection, will be eligible for entry into this trial. Patients must have failed treatment with at lease one fluoropyrimidine regimen. Patients will be treated on one of two schedules. On schedule A, the recombinant DNA will be administered in escalating doses to groups of 3 patients at each of 3 dose levels: 10, 50, and 250 μg. On schedule A, each patient will receive only a single injection. Each of 3 patients will be observed for toxicity and response for 30 days before patients will receive DNA at the next planned higher dose, 50 μg. The same procedure will be followed at this dose level before proceeding to the final dose level, 250 μg. Schedule B will be initiated in new patients after all 3 patients given 50μg DNA have been observed and found not to have unacceptable toxicity. On schedule B, 1 group of 3 patients will receive 10μg DNA days 1 and 15. If at 30 days no patient has unacceptable toxicity, a second group of 3 new patients will receive 10μg DNA on days 1, 15, and 30.

2.0 Goals

2.1 To determine safety and toxicity of direct intralesional injection of increasing amounts of a DNA/lipid mixture: VCL-1005 (HLA-B7/DMRIE/DOPE) into solid tumors in selected patients with metastatic colorectal adenocarcinoma. Escalating treatment regimens will be used and tumor growth evaluated.

2.2 To measure the cytotoxic T-cell activity directed towards antigens on tumor cells other than HLA-B7.

2.3 To measure humoral and cellular immune responses to HLA-B7.
2.4 To confirm expression in vivo of the HLA-B7 gene in the tumor cells.
2.5 To characterize the clinical response to escalating doses of the study drug by assessing the size of the injected tumor and of other tumor masses that may be present.

3.0 Patient Eligibility

3.1 Required characteristics -- Prior to discussing protocol entry with the patient, call the Randomization Center to insure that a place on the protocol is open to the patient.

3.1.1 Must have histologically-confirmed metastases from primary adenocarcinoma taking origin in the colon or rectum.

3.1.2 Must have at least two liver metastases that are clearly measurable in two dimensions on CT scan, one of which must measure between 2.0 and 4.0 cm in greatest diameter and would be accessible for intralesional injection.

3.1.3 Must have had either prior standard therapies for their disease and have become unresponsive to them or have made the decision that other therapy would not be of any benefit.

3.1.4 Must be $\supseteq$ 18 years old.

3.1.5 The following laboratory values (<15 days of registration):

- white blood count >3,000.
- platelet count >100,000.
- Hemoglobin >9g/dl.
- prothrombin time >1 second greater than control.
- serum creatinine >125% of upper limit of normal.
- direct serum bilirubin above the upper limit of normal or
- SGOT or alkaline phosphate >3x above the upper limit of normal.

3.1.6 Must have a baseline Karnofsky Performance Scale (KPS) score of at least 70.

3.1.7 Estimated life expectancy of at least 12 weeks.

3.1.8 HLA typing must indicate that the patient is HLA-B7 negative.

3.1.9a Must be HBsAg and anti-HIV antibody negative since positivity for either disease may be associated with reduced immune competence.

3.1.9b Women/men of reproductive potential agree to use effective contraception.

3.1.10 Patients must be immunocompetent by having at least 2/7 positive skin tests on the Merieux Multitest and by having a PHA lymphocyte response in the normal range.

3.2 Contraindications

3.2.1 Positive antibody to HIV or HBsAg.

3.2.2 Active autoimmune disease.
3.2.3 Hepatitis (acute or chronic active).
3.2.4 Active infection.
3.2.5 The following therapies within the past three weeks:
- radiation,
- chemotherapy,
- steroidal (glucocorticsteroids).

3.2.6 Diabetes mellitus not controlled by medical treatment.
3.2.7 Psychiatric illness that may make compliance to the clinical protocol unmanageable or may compromise the patient's ability to give informed consent.
3.2.8 Immunoincomptetence as defined in eligibility criteria.

4.0 Test Schedule - See Appendix

5.0 Stratification Factors - None.

6.0 Registration/Randomization Procedure

6.1 Prior to discussing protocol entry with the patient, call the Randomization Center to insure that a place on the protocol is open to the patient.
6.2 To register a patient, call (4-2753) or fax (4-0885) the Randomization Center between 8 a.m. and 5 p.m. central time Monday through Friday.
6.3 A signed HHS 596 form must be on file in the Randomization center before an institution may register any patients.
6.4 Patient eligibility and the existence of a signed consent form will be checked by Randomization Center personnel before a patient will be registered into this study. Dose level will be given.
6.5 Treatment on this protocol must be given at the Mayo Clinic-Rochester on an impatient basis under the supervision of a medical oncologist. Actual intralesional injection of tumor will be carried out by Dr. J. William Charboneau or Dr. Carl Reading, Department of Diagnostic Radiology.
6.6 Treatment must begin within 15 days of registration.
6.7 Pre treatment tests must be completed within the guidelines specified on the test schedule.

7.0 Protocol Treatment

7.1 Patients will be admitted to the General Cancer Research Center (GCRC) at Saint Mary's Hospital prior to each intralesional injection.
7.2 Identification and localization of a specific liver metastasis for injection using hepatic ultrasound meeting the criteria stated in section 3.0 will be carried out by Drs. Charboneau or Reading.
7.3 The appropriate volume of DNA/lipid mixture prepared in Saint Mary's Pharmacy will be injected into the pre-selected hepatic metastasis on day 1. The injection is done by the diagnostic radiologist using standard

procedures in the Diagnostic Radiology Suite at Saint Mary's Hospital. The needle is inserted under direct sonographic visualization. A constant volume of 4ml lipid preparation will be used at all DNA concentrations.

7.4 Amounts of DNA in 4ml of lipid to be injected:

Schedule A

Group	Number of Patients	µg of DNA
A-1	3	10µg day 1 only
A-2	3	50µg day 1 only
A-3	3	250µg day 1 only

Schedule B

(Will be initiated after safety of schedule A at 50µg has been established)

Group	Number of Patients	µg of DNA
B-1	3	10µg day 1 and 15
B-2	3	10µg day 1, 15 and 30

7.5 Criteria for re-treatment: Patients with a partial response at 4 weeks after the last injection of their initial course may receive an additional course of treatment identical to the first treatment, i.e., on Schedule A, at 4 weeks a responding patient may receive a second injection at the same dose initially given; on Schedule B, 4 weeks after two injections (B-1) and 4 weeks after three injections (B-2), responding patients may receive a second course identical to their first course.

7.6 The primary objectives of this investigation are to determine the feasibility and safety of intralesional injections of DNA/lipids into liver metastases. Patients will be entered in groups of 3 at each dose level and observed for at least 4 weeks before entry of additional patients.

Number of patients with ⊇ grade 3 toxicity (World Health Organization [WHO])	Instructions for entry of additional patients
0	Escalate to next volume level.
1	Enter 3 more patients at same volume level.
2 or 3	Discontinue further patient entry at that level and enter 3 more patients at the previous level.

8.0 Treatment Modification Based on Toxicity

Toxicity	Change
SGOT or alk phos >3x UNL and ⊇50% ↑ compared to preRx baseline Direct bili ⊇0.2mg/dl above UNL	
Other Rx complications or medical conditions which substantially ↑ risk to patient	No further Rx
Symptomatic tumor PROG requiring other Rx e.g. RT or chemo	

9.0 Ancillary Treatment

Symptomatic care may be given as required with medications such as antiemetics and analgesics. However, administration of corticosteroids will require that the patient be removed from study.

10.0 Toxicity Monitoring and Adverse Reaction Reporting

Patients will be monitored and questioned at every visit (see Schedule of Events) regarding the occurrence and nature of any adverse experiences. An event is any change in the physiological or psychological state other than the primary condition that qualifies the patient for this study.

The Investigator must report to Mr. Steven Kradjian (ph 619-453-9777, available 24 hours.) upon occurrence of any life-threatening events (Grade IV) that may be attributable to administration of the study drug, all fatal events, or the first occurrence of any previously unknown clinical event (regardless of Grade). A written report is to follow within 3 working days to:

Steven A. Kradjian, Director
Regulatory Affairs
Vical Inc.
9373 Towne Centre Drive, Suite 100
San Diego, CA 92121

Patients will be taken off study immediately if :

Unacceptable toxicity (Grade III or IV) develops and is not easily corrected (refer to WHO toxicity sheet).

Development of Progressive Disease (see definition) requiring the institution of alternative treatments such as radiation, surgery or other drug therapy. If the Investigator believes that the patient's best interest requires a change of therapy.

At the patient's or guardian's request.

10.1 Toxicities to be graded at each evaluation and pre-treatment symptoms/conditions to be evaluated at baseline per WHO Criteria:

Toxicity/symptoms	Baseline	Each Evaluation
Fatigue	X	X
Weight loss/anorexia	X	X
Nausea	X	X
Vomiting	X	X
Hemorrhage		X
Infection		X
Liver (SGOT, alk phos, and total bilirubin)	X	X

11.0 Treatment Evaluation

11.1 Patients will be evaluated by a Mayo Clinic medical oncologist at least every 4 weeks while receiving treatment, every 8 weeks until there is evidence of tumor progression, and then 8-12 week intervals thereafter as long as the patient is able to return to the Mayo Clinic.

11.2 Evaluation of tumor regression is a secondary endpoint in this clinical trial. The following criteria will apply to measurable indicator lesions within the liver or for any measurable extrahepatic lesions that may be present.

11.3 Minimum size requirements for measurable indicator lesions:

11.3.1 Tumor masses with clearly defined bidimensional measurements. The minimum size of the indicator lesion depends on the method of measurement as follows:

Method of Measurement	Minimum Size of Largest Tumor Diameter
Physical examination or chest x-ray (discrete lesion that can be measured with calipers)	1.0cm
CT Scan*	3.0cm

*Note: Special attention should be paid to identify the specific lesion being measured, and to define an anatomic landmark to identify the level of the cross-sectional image to facilitate serial measurements. Lesions 2.0-3.0 cm in diameter may be used as indicator lesions if serial images are obtained at 0.5 cm intervals through the tumor.

11.4 Criteria for a partial response (PR)

11.4.1 bidimensional indicator lesion(s)

≥50% reduction in the sum of the products of the largest perpendicular diameters of the indicator lesion(s), single or multiple sites, chosen prior to therapy. Response status will be determined separately for the injected tumor nodule and other measurable lesions that are not injected.

11.5 Criteria for complete response (CR)

Total disappearance of all evidence of tumor.

For a patient to qualify for complete response or partial response, none of the factors constituting progression may be present (see below).

11.6 Criteria for progression (PROG)

Tumor progression will be declared if one or more of the following criteria are met.

11.6.1 Appearance of new lesion(s).

11.6.2 Increase in tumor size.

11.6.2.1 Patients with measurable indicator lesion(s) who have met the criteria for partial response.

Significant increase in the size of indicator lesion(s) compared to the smallest measurements while on study.

Progression will be declared when the indicator lesion has increased in size from the smallest measurement by at least 50% of the decrease in size between pre-treatment measurements and smallest measurement at the point of maximum tumor reduction.

11.6.2.2 Patients with measurable indicator lesion(s) who have met the criteria for complete response:

Progression will be declared if there is reappearance of any tumor.

11.6.2.3 Patients with measurable indicator lesion(s) compared to pre-treatment measurements.

11.6.3 Significant clinical deterioration that cannot be attributed to treatment or other medical conditions.

11.6.3.1 Weight loss >5% body weight.

11.6.3.2 Worsening of tumor-related symptoms.

11.6.3.3 Decline in performance status >1 level on ECOG scale.

11.7 Criteria for stable (STAB)

Failure to meet the criteria for complete response, partial response, regression, or progression.

12.0 Descriptive Factors - None

13.0 Treatment/Follow-up Decision at Evaluation of Patient

Patients meeting the criteria for partial response at 4 weeks after their single injection on Schedule A or after their last injection on Schedule B may be retreated once.

14.0 Pharmacologic/Immunologic Studies

14.1 Immunochemical staining will be done in the Immunohistochemical Core Laboratory of the Mayo Cancer Center under the direction of Dr. Patrick Roche. Pre- and post-treatment tumor cells are stained with anti-HLA-B7 antibodies, ME-1, BB7.1, and GSP5.3 (G. Nabel, personal communication) to look for expression.

14.2 Presence of DNA from the HLA-B7 gene will be assessed by PCR amplification of cells obtained by biopsy of the treated site on days 8, 15, and 29 after injection of DNA/lipid complex. Genomic DNA is isolated by standard methods and a portion of the HLA-B7 gene is amplified and sequenced. Several primer sequences may be used (G. Nabel, personal communication).

14.3 Development of circulating antibodies to HLA-B7 will be evaluated. Autologous peripheral blood B lymphocytes will be EBV immortalized and subjected to *in vitro* gene transfer with the DNA/lipid complex. these autologous cells expressing the HLA-B7 gene will be used to assess the specificity of antibody response to the *in vivo* transfer of the gene. Evidence of cytolytic T-cells will be assessed if a sufficient amount of material is available for successful expansion of infiltrating T-cell population from cells in biopsy of the metastasis. These studies will be carried out in the Cellular Immunology Laboratory under the direction of Dr. Homburger.

15.0 Drug Information

The study drug (VCL-1005) will be supplied by Vical as two sterile vials containing (i) HLA-B7 plasmid DNA, and (ii) DMRIE/DOPE lipid mixture. Diluent will be lactated Ringer's. All components will be stable for at least eight weeks under recommended storage conditions (DNA -20°C, DMRIE/DOPE 4°C). The materials will be supplied by Vical Inc.

This study drug is composed of plasmid DNA coding for the complete human MHC HLA-B7 formulated with the cationic lipid mixture DMRIE/DOPE (lipid complex formulation). The DNA concentration is 1.0 mg/ml (see Investigator's brochure for complete details of product characteristics and preparation). Lactated Ringer's is readily available at the site.

DNA/lipid complexes are prepared immediately prior to administration. DNA is supplied in 1.0 mg/ml concentration in 400 µl lactated Ringer's solution. Lipid (DMRIE/DOPE) is supplied as a dried film. Each vial contains 77 µg DMRIE and 90 µg DOPE. Each vial is reconstituted with 400 µl lactated Ringer's solutions by vortexing until homogeneous. The contents of the lipid vial is transferred into the

DNA vial and mixed well by repeated inversion. The final concentration of the HLA-B7 plasmid DNA is 500 µg/ml. The amount injected into each tumor will be 0.5 ml, for the highest dose of 250 µg. Lower doses, 10 and 50 µg, will be prepared in a similar fashion or formulated as dilutions with lactated Ringer's.

Tumor lesions will be selected for treatment if they are accessible to intratumor administration by direct needle injection or intravascular catheter. These metastatic lesions will be located in the liver for colorectal adenocarcinoma. The study drug will be injected with the aid of sonographic visualization of the metastasis.

Prior to injection following placement of the needle, gentle aspiration will be applied to the syringe to ensure that no material is injected intravenously. Vital signs will be measured every 15 minutes prior to, during, and after the injection for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80mm Hg, the injection will be terminated immediately, and the patient will be closely monitored until blood pressure is normalized.

Immediately after the injection, a blood sample will be obtained to check serum enzymes, blood chemistries and cell counts, and to analyze by PCR for the presence of HLA-B7 Plasmid DNA in the peripheral blood. Every patient will be observed for 48 hours and another blood collection will be drawn. If there are no complications, the patient will be discharged. If any abnormalities appear, the patient will be closely observed. All toxicities will be graded according to the WHO recommendations (see Appendix 2).

16.0 Statistical Consideration and Methodology - Descriptive statistics only will be performed due to the small number of patients.

17.0 Pathology Considerations

17.1 Patients entering this trial will already have had histologically-documented metastatic colon carcinoma. Liver biopsy is carried out as part of this protocol including the initial one to demonstrate unequivocally by histologic examination of a frozen section that the metastasis selected for injection of the DNA/lipid complex is, in fact, metastatic cancer. Biopsy samples are never to be placed in fixative. Jill Piens, R.N. (507/284-4911, or pager 4-6362) to call Dr. J.S. Kovach's laboratory and state that a biopsy sample on this immunotherapy protocol has been obtained and is being held in a dry sterile container placed on ice to be picked up.

17.2 For biopsies in which a core of tumor has been obtained, the sample will be subdivided and at least half frozen in liquid nitrogen. The remainder

may be subjected to tissue disruption in an attempt to obtain and expand T-cells present in the tissue.

18.0 Records and Data Collection Procedures

18.1 Mayo Oncology records

18.1.1 The Oncology Records will be utilized for patients. Data will be entered into the computer within 3 weeks after each evaluation of the patient. After the patient goes off treatment, follow-up information will be collected and entered into the computer every 3 months.

18.1.2 Pathology diagnosis made by frozen section is reported in a conventional way with a record being placed in the patient's permanent medical record.

18.1.3 Data from the Cellular Immunology Laboratory may be entered into their computer using the patient's clinic number as a key. Summary reports of samples received will be sent to the principal investigator monthly.

18.2 Vical records

Case report forms, regulatory documents and study source documents will collected and reviewed in conformance with FDA Good Clinical Practices.

Test Schedule

Tests and Procedures	<15 days prior to registration	During course of Rx days 3, 15, and 29[7]	Follow-up: at 8 wks post Rx and q 8 wks until PROG, then q 8-12 wks
History and exam, tumor meas., ht, wt, PS	X	X	X
Vital signs (pulse, blood pressure, temperature)		X[1]	
Hematology group WBC, HgB, PLT	X	X	X
Chemistry group (SGOT, alk. phos., dir. and t. bili, creat.)	X	X	X
PT, Ptt	X	Days 15 and 29 only	Day 57[7]
Electrocardiogram	X		
Chest x-ray	X		
CT scan & ultrasound	X	Days 15 and 29 only	X
Tumor biopsy-guided by hepatic ultrasound		Days 15 and 29 only	Days 43[5,7] and 57[5,7]
Urinalysis	X		
HbAg, HIV titer	X		
HLA-B7 phenotype	X		
Assay for cytoxic T-cells and antibody[2]		X	X
Peripheral blood sample	X[3]	X[4]	Day 57[7]
Antinuclear antibodies	X	Day 29 only	Day 57[7]
Serum preg. test[6]	X		

1. Prior to each biopsy and treatment and them every 15 minutes until stable.

2. Biopsy tissue will be assayed for the presence of the HLA-B7 gene by PCR. Expression of the HLA-B7 gene will be looked for immunohistochemically. When sufficient materials are present, attempts to assess the development of cytolytic T-cell response to the cells of the tumor will be evaluated. Tissue will be "snap" frozen in liquid nitrogen (LN) and sent in LN to Dr. P.CX. Roche, Immunhistochemical Core Lab, Hilton Building.

3. 45ml of clotted blood for serum storage and 45ml of anti coagulated blood for mononuclear cell cryopreservation will be obtained (sent at room temperature to Dr. H.A. Homburger, Cellular Immunology core Laboratory, Hilton Building).

4. 20ml of clotted blood will be obtained to look for development of antibodies, and 20ml of anti coagulated blood for mononuclear cell cryopreservation will be obtained (sent at room temperature to Dr. H.A. Homburger, Cellular Immunology Core Laboratory, Hilton Building).

5. Only for patients receiving 2 or 3 injections of DNA on schedule B.

6. For women of childbearing potential only. Must be done within 7 days prior to registration.

7. Days 29, 43, and 57 are approximate. The goal will be to obtain the studies as close as possible to 4, 6, and 8 weeks +/- 2 days.

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Title: Phase I Study of Advanced Colorectal Carcinoma by Direct Gene Therapy into Hepatic Metastases

Participants: Joseph Rubin, MD, J. William Charboneau, MD Carl Reading, MD, John S. Kovach, MD

PATIENT CONSENT FORM

FEDERAL REGULATIONS REQUIRE WRITTEN INFORMED CONSENT FROM PARTICIPANTS PRIOR TO PARTICIPATION IN A RESEARCH STUDY SO THAT THEY CAN KNOW THE NATURE AND RISKS OF PARTICIPATION AND CAN DECIDE TO PARTICIPATE OR NOT TO PARTICIPATE IN A FREE AND INFORMED MANNER. YOU ARE ASKED TO READ THE FOLLOWING MATERIAL TO ENSURE THAT YOU ARE INFORMED OF THE NATURE OF THIS RESEARCH STUDY AND OF HOW YOU WILL PARTICIPATE IN IT IF YOU CONSENT TO DO SO. SIGNING THIS FORM WILL INDICATE THAT YOU HAVE BEEN SO INFORMED AND THAT YOU GIVE YOUR CONSENT.

The purpose of this study is to determine the safety and optimal dose of a DNA (deoxyribonucleic acid)/liposome compound when injected directly into colorectal cancers which have spread to the liver. The DNA/liposome complex contains a human gene (referred to as HLA-B7) which is known to enhance rejection of foreign cells by the immune system in individuals who do not carry this gene naturally. The goal of this study is to transfer this gene to the cancer cells in the liver, thereby making them (and possibly any malignant cells elsewhere in the body) susceptible to destruction by the normal immune cells (cytotoxic lymphocytes) of the patient. The chance of benefit from this experimental therapy cannot be predicted.

Participants in this study will be admitted to the General Clinical Research Center (GCRC) at Saint Marys Hospital. Up to four direct injections of the DNA/liposome complex into a single liver tumor will be performed in the Diagnostic Radiology Department at Saint Marys Hospital using an ultrasound technique to directly visualize the tumor. A biopsy of the tumor will be obtained immediately prior to the first injection. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and multiple areas within a single nodule will be injected up to 4 times. The duration of this procedure is usually 30 minutes. Participants will be observed for 24-48 hours in the GCRC following the injection. Biopsies of the liver nodule will be repeated at Saint Marys Hospital two weeks and four weeks after injection of the tumor. If there is evidence that the tumor is shrinking four weeks following injection, the participant may receive one or two additional injections into the liver at 4-week intervals. These additional injections will be considered on a case-by-case basis only, following consultation with the patient-subject, physician-researchers, and the sponsor. If uninjected tumor nodules elsewhere in the body also shrink, a needle biopsy of these tumors may also be done to study the mechanism of tumor destruction. Blood samples for research purposes will be obtained prior to tumor injection at 1, 2, 4, 8 weeks, then every 3 months for 1 year. The total volume of blood drawn for research purposes will be less than 500 mL (one pint).

No side effects other than local inflammation have been observed in preliminary studies of direct injection of a similar DNA/liposome complex into skin tumors in patients with malignant melanoma. There is a theoretical possibility that inflammation of normal body tissue producing symptoms such as joint pain, skin rash, or damage to the kidneys could occur in response to transfer of the HLA-B7 gene to the tumor. There is also the potential risk of damage to the liver if severe inflammation occurs around the injected liver tumor. Bleeding and infection are rare complications associated with needle biopsies of tumors in the liver or elsewhere within the body. All care will be taken to minimize the risk of complication, although the nature and severity of side effects is unpredictable.

Although there is no evidence that the gene transferred to the tumor cells can also enter egg cells or sperm cells, this is a theoretical possibility. Therefore, this study may be hazardous to an unborn child. There is no information as to whether there are significant risks to a fetus carried by a mother who is exposed to the DNA/liposome complex. Therefore, only females who are postmenopausal (no longer menstruating), and men and women who are surgically sterilized, or agree to use effective means of contraception may participate in this study.

Alternative interventions for this type of malignant disease in the liver spreading from a cancer of the colon or rectum would consist of investigative chemotherapy or symptomatic care. There is no known cure for this condition. A physician will discuss these alternative approaches in detail as they may relate to the patient's decision to participate.

Participants in this investigational program, as in most cancer research, undergo periodic monitoring for signs of change in the condition of their tumors, for any toxic effects of the research intervention, and to allow the physician-researchers to adjust what they are doing. Such monitoring includes physical examinations and studies such as x-rays, scans, and blood tests. All such procedures carried out for research purposes, as well as room and board and nursing charges at the General Clinical Research Center, will not be billed to patient-subjects in this study. Procedures that are solely for treatment purposes, as well as pharmacy charges and other ancillary expenses such as oral medications prescribed at the time of discharge, will be billed to the patient-subjects and/or their insurers.

The principal investigator, Dr. Joseph Rubin, or a co-investigator, telephone 284-4718, may be contacted at any time if the participant has any questions regarding this study, if further information about the nature and conduct of the study is required, or if any problems result from participation, including a research-related illness. Participation in this research study is voluntary and the participant may refuse to enter the study or may discontinue participation in the study at any time without jeopardizing present or future medical care and treatment or other benefits to which the participant is entitled. The investigators may discontinue the participant's study involvement at any time if it is felt to be in the participant's best interest, if the participant does not comply with the study requirements, or if the study is stopped. Participants will be informed of any changes in the nature of the study or in the procedures described if they occur.

No commitment is made to provide free medical care or compensation for any adverse results because of participation in this study. Medical services will be offered at the usual charge. Further information concerning policies in this regard or information about the conduct of this study or the rights of research subjects, may be obtained from IRB or Legal Department, telephone 284-2511.

Although data from this study may be published, confidentiality of information concerning participants will be maintained. Names of participants or material identifying participants will not be released without written permission except as such release is required by law. Medical records related to this study may be made available to the National Institutes of Health, Food and Drug Administration, or the sponsor as provided in federal regulations.

I HAVE HAD AN OPPORTUNITY TO HAVE MY QUESTIONS ANSWERED. A COPY OF THIS FORM HAS BEEN GIVEN TO ME. I AGREE TO PARTICIPATE IN THIS MEDICAL RESEARCH STUDY UNDER THE DIRECTION OF THE PRINCIPAL INVESTIGATOR AS LISTED ABOVE.

_____ _____

(Date) (Signature of Participant)

_____ _____

(Date) (Signature of Investigator Obtaining Consent)

BIOGRAPHICAL SKETCH

CVs

Give the following information for the key personnel and consultants listed on page 2. Begin with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	POSITION TITLE	BIRTHDATE (Mo., Day, Yr.)
Joseph Rubin, M.D.	M.D.	3-26-42

EDUCATION (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Emory University, Atlanta, Georgia	B.A.	1963	Medicine
Medical College of South Carolina Charleston, South Carolina	M.D.	1967	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1967 - 1968 Cook County Hospital; Chicago, Illinois; Internship (Rotating Medical)
1970 - 1972 Mayo Graduate School of Medicine; Rochester, Minnesota; Internal Medicine
1972 - 1974 Mayo Graduate School of Medicine; Rochester, Minnesota; Hematology Residen
1974 - 1976 Mayo Graduate School of Medicine; Rochester, Minnesota; Oncology Residency

Military Service:
1968 - 1970 United States Navy

Certification and Licensure
1967 Licensure in South Carolina
1970 Licensure in Minnesota
1975 American Board of Internal Medicine
1977 Board Certified in Subspecialty of Medical Oncology

Professional Experience:
1975 - 1976 Associate Consultant; Division of Medical Oncology; Mayo Clinic; Rochester, Minnesota
1976 - Pres Consultant; Division of Medical Oncology; Mayo Clinic; Rochester, Minnesota
1976 - 1986 Program Director, Division of Medical Oncology; Mayo Clinic; Rochester, Minnesota
1986 - Pres Chairman; Division of Medical Oncology; Mayo Clinic; Rochester, Minnesota

Educational Experience:
1976 - 1981 Instructor, Mayo Medical School; Rochester, Minnesota
1981 - 1985 Assistant Professor of Oncology; Mayo Medical School; Rochester, Minnesota
1985 - Pres Associate Professor of Oncology; Mayo Medical School; Rochester, Minnesota

DUPLICATE COPY - USE IF NEEDED

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9312-065 Chang, Alfred E.; University of Michigan, Ann Arbor, Michigan; *Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the IL-4 Gene.*

Date of RAC Approval: 12-3-93
Date of NIH Approval: 8-23-94

SCIENTIFIC ABSTRACT

Although it has been well established experimentally that the transfer of sensitized T lymphocytes can mediate potent antitumor effects, extrapolating the principles of adoptive immunotherapy obtained from animal studies to clinical therapy will require the development of innovative techniques to isolate and propagate antitumor effector T cells from cancer patients. Toward this end, we have established culture methods whereby cells from tumor-draining or tumor primed lymph nodes (LN) can be sensitized to acquire therapeutic efficacy. Since these cells do not demonstrate overt antitumor reactivity before culture, they are functionally referred to as "pre-effector" cells. One method to generate antitumor effector T cells involves the sequential activation of pre-effector LN cells with anti-CD3 mAb followed by expansion in low concentrations of IL-2. Animal experiments have demonstrated that the antitumor reactivity of these anti-CD3/IL-2 activated cells are exquisitely tumor-specific and mediate the regression of established tumor in adoptive immunotherapy.

A major obstacle which confronts the clinical application of adoptive immunotherapy is the relatively weak immunogenicity of human cancers which hampers the induction of sensitized pre-effector cells. Recent observations in animal studies indicate the tumors can be genetically altered to enhance the host immune response against native or parental tumor antigens. The transfection of tumor cells with some cytokine genes have often resulted in T cell mediated rejection of these tumors by their syngeneic hosts. Similarly, we found that the transfection of the poorly immunogenic B16BL6 murine melanoma tumor with the IL-4 gene resulted in decreased tumorigenicity when inoculated into the syngeneic host. More importantly, draining LN cells removed from these animals and activated by the anti-CD3/IL-2 culture procedure generated potent therapeutic effector cells which mediated the adoptive immunotherapy of established metastatic parental tumors. These observations provide the rationale for this clinical protocol to examine autologous tumor cells modified with the IL-4 gene, which will be utilized as a vaccine to induce pre-effector LN cells in patients with metastatic melanoma. These vaccine-primed LN cells will be activated by the anti-CD3/IL-2 method and subsequently transferred intravenously to patients along with the concomitant administration of IL-2 (360,000 IU/kg q8h x 5 days) to support their survival/function in vivo.

The specific aims of the protocol are: 1) To assess the feasibility and toxicity of adoptive T cell immunotherapy of melanoma with anti-CD3/IL-2 activated LN cells that are primed in vivo with IL-4 modified autologous tumor cells, 2) To evaluate the antitumor efficacy and in vivo immunological reactivity of patients receiving adoptively transferred T cells, and 3) To investigate the in vitro immunological reactivities of the activated T cells that might correlate with their in vivo antitumor function.

NON-TECHNICAL ABSTRACT

This study involves the use of novel methods to treat patients with metastatic melanoma by manipulation of their immune system. Based on extensive animal studies, we know that the immune system has the potential to recognize tumor cells as being foreign to the body and destroy them. The critical component of the immune system which appears to be involved in the rejection of tumors are lymphocytes. The ability to generate these lymphocytes in the laboratory would be useful for potential therapy of cancer. This approach has been called adoptive immunotherapy and involves the infusion of cancer reactive lymphocytes into patients in order to cause tumor shrinkage.

Unfortunately, the generation of immune lymphocytes which can reject human cancers has been extremely difficult. One possible reason why this problem exists is that the foreign proteins present on human tumor cells are very weak in their ability to stimulate the immune system to react against them. Based on our animal studies, we have devised an approach to artificially stimulate immune cells that may be used for the treatment of human tumors that under normal circumstances would not occur. This involves a two-step process. The first step requires vaccination of the patient with their own tumor cells which have previously been removed and genetically engineered to secrete an immune factor called interleukin 4 (IL-4). The tumor cells are irradiated prior to injection into the skin in order to prevent outgrowth of tumor at the site. It has been found in animal studies that the production of IL-4 by the gene-modified tumor cells promotes an immune response in lymph nodes near the vaccination site. Lymph nodes are small glands of the immune system where lymphocytes congregate. The lymph nodes adjacent to the vaccination sites will be surgically removed approximately 7 to 10 days later and taken to the laboratory for further processing. In the laboratory, cells from the lymph nodes will be stimulated and grown in special flasks by methods we have previously described for a 2 to 3 week period in order to generate a large number of immune lymphocytes. These lymphocytes will be collected and infused back into the patient along with the administration of interleukin-2, another immune protein. The interleukin-2 has been found to promote the antitumor effect of the immune lymphocytes in cancer patients.

This clinical study proposes to address several important questions. These questions include: 1) Can this clinical treatment program be performed as described and what are its side-effects, 2) What antitumor response can be seen with this treatment, and 3) What is the immunological function of the immune lymphocytes, as assessed by laboratory tests.

HUMAN GENE THERAPY PROTOCOL:

Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the IL-4 Gene

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Submitted to RAC: October 7, 1993

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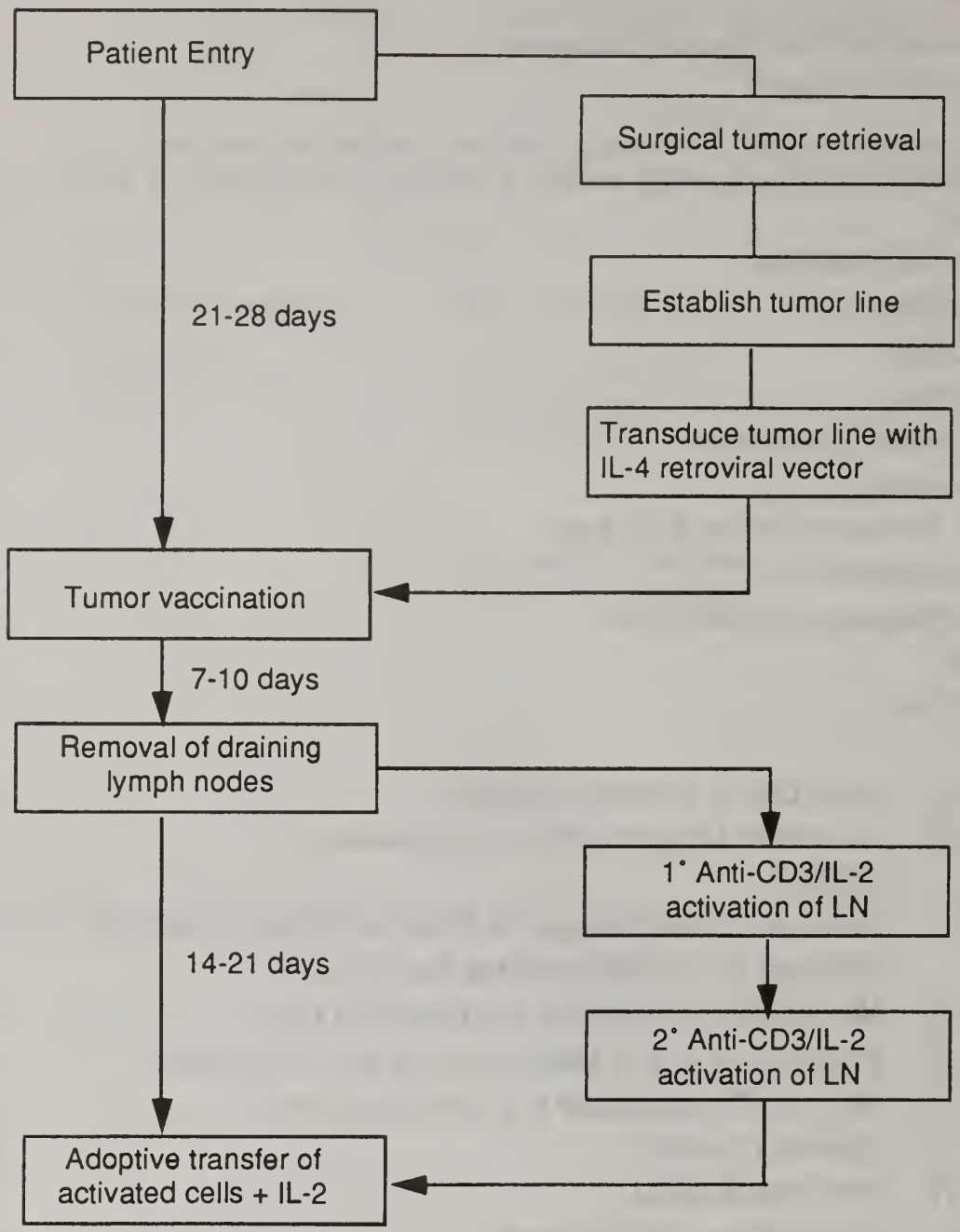
PROTOCOL: Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed in Vivo with Autologous Tumor Cells Transduced with the IL-4 Gene

1.0 Protocol Schema



SCHEMA: Patients with advanced melanoma will undergo surgical retrieval of tumor for use as a tumor vaccine. A tumor cell line will be established in culture and subsequently transduced with a retroviral vector containing the IL-4 gene. The transduced line will be screened for IL-4 production and subsequently irradiated with 5,000 cGy. Patients will be vaccinated with 10^7-10^8 irradiated transduced tumor cells intradermally in 1 or 2 sites. Surgical removal of draining lymph nodes (LN) 7 to 10 days after vaccination will be

performed to obtain lymphocytes for activation with anti-CD3 monoclonal antibody (OKT3) and expansion in IL-2 in vitro. After in vitro activation, these lymphocytes will be adoptively transferred intravenously to the patient followed by the administration of IL-2 (360,000 IU i.v. every 8 h. for 5 days). Fifteen patients will be treated to gain sufficient information regarding toxicity, antitumor reactivity and immunologic function.

2.0 OBJECTIVES

Our laboratory has described a new method of generating sensitized T cells for adoptive immunotherapy utilizing autologous tumor cells genetically modified to secrete IL-4. In preclinical studies, the in vivo administration of these tumor cells has been found to elicit sensitized T cells in the draining lymph nodes (LN) against a poorly immunogenic tumor. The subsequent in vitro activation of these LN cells by anti-CD3 mAb and IL-2 has resulted in the generation of T cells capable of mediating regression of established parental tumor metastases. These preclinical studies have formed the basis for a clinical evaluation of similarly derived T cells for patients with advanced melanoma. The objectives of this study are:

2.1 To assess the feasibility and toxicity of adoptive T cell immunotherapy of melanoma with anti-CD3/IL-2 activated LN cells that are primed in vivo with IL-4 modified tumor cells.

2.2 To evaluate the antitumor efficacy and in vivo immunological reactivity of patients receiving adoptively transferred T cells.

2.3 To investigate the in vitro immunological reactivities of the activated T cells that might correlate with their in vivo antitumor function.

3.0 BACKGROUND AND RATIONALE

Adoptive immunotherapy is defined as the transfer of antitumor reactive cells to the tumor-bearing host which will directly or indirectly mediate the regression of tumor. The therapeutic efficacy of adoptive immunotherapy of cancer with sensitized T lymphocytes is well-documented in animal studies. The application of this approach for the treatment of human cancer poses many theoretical and technical difficulties since it requires the generation of large quantities of antitumor reactive lymphocytes from cancer patients. Despite such limitations, many observations established in animal models of adoptive immunotherapy have been implemented in clinical trials with encouraging results. The demonstration of regression of solid tumors in man following the adoptive transfer of lymphokine-activated killer (LAK) cells or tumor-infiltrating lymphocytes (TIL) in conjunction with IL-2 has established that cellular therapy is a feasible alternative in treating cancers and has encouraged the search for more potent effector cells (1,2).

3.1 Animal studies characterizing the immune T cell response in tumor-draining LN

In spite of the demonstration that many animal tumors possess tumor-specific antigens which are capable of immunizing syngeneic hosts to reject a challenge of an otherwise lethal dose of tumor cells, antigenic tumors often grow in their immunocompetent hosts. Apparently, during progressive tumor growth, the innate immune response is insufficient due to the weakness of the antigen and/or the occurrence of tumor-induced immunosuppression. Using several immunogenic tumors, we have demonstrated that LN draining a progressively

growing tumor contain T cells that were immunologically sensitized but functionally deficient in expressing antitumor reactivity. This population of cells designated as "pre-effector" cells could nevertheless be further stimulated in vitro to differentiate into potent immune effector cells (3,4). Initially an in vitro sensitization (IVS) procedure was used for the generation of effector cells by the culture of tumor-draining LN cells with excessive numbers of irradiated tumor cells in the presence of IL-2. In IVS, the function of tumor cells was to provide specific antigenic stimulation while IL-2 promoted proliferation of T lymphocytes. In extensive animal studies with the immunogenic MCA 105 and MCA 106 sarcoma tumors, we have documented that IVS cells generated from tumor-bearing mice were capable of curing a significant proportion of animals with grossly visible metastatic disease in the lung and liver (5).

The success of adoptive immunotherapy was critically dependent upon the immunogenicity of the tumor. In animal studies, therapeutic IVS cells could only be generated when the tumors were immunogenic and capable of eliciting transplantation resistance in their syngeneic hosts. It has not been possible to apply this approach to tumors which do not induce systemic immunity. A major concern for cancer immunotherapy in humans is whether or not immunogenic animal tumors represent sufficient models because human tumors originate spontaneously and may be poorly immunogenic. To address this issue, we examined the feasibility of generating immune effector cells with reactivity against poorly immunogenic murine tumors (6,7). The MCA 102 was selected for study because it failed to elicit systemic immunity in syngeneic B6 mice by a variety of immunization procedures including tumor growth and excision, immunization with irradiated tumor cells and with various combination of tumor cells admixed with C. parvum. Initial experiments with the MCA 102 tumor indicated that unlike the MCA 105 and MCA 106 tumors, therapeutic effector cells could not be induced from lymph nodes draining a progressive tumor. However, utilizing alternative methods, we found it was possible to generate sensitized lymphocytes with therapeutic efficacy against the MCA 102 tumor. The procedure to achieve this required both *in vivo* tumor priming and IVS. Unlike pre-effector cells sensitized to the weakly immunogenic MCA 105 tumor which were elicited naturally during progressive tumor growth, the process of inducing pre-effector cells to the MCA 102 tumor was an artificial one and required the use of a bacterial adjuvant, C. parvum, in addition to tumor cells administered as a vaccine. If properly primed pre-effector cells are induced, the IVS method can provide an environment for them to mature into functional immune cells. Indeed, using this approach, transfer of as few as 1.5×10^7 vaccine-primed IVS cells not only reduced established pulmonary MCA 102 metastases but also prolonged survival and cured tumors in a majority of treated animals (6). ***Analysis of the distribution of pre-effector cells in various lymphoid organs revealed that their occurrence was regionally restricted and only detectable in lymph nodes draining the tumor vaccine.*** During the peak response, no evidence of the existence of pre-effector cells in the spleen, mesenteric lymph nodes, PBL or bone marrow was seen. Based on these studies, we have completed a clinical study to evaluate the immunobiology and efficacy of similarly generated primed LN cells in patients with advanced cancer (see Section 3.2).

The requirement for a relatively large number of autologous tumor cells for generating IVS cells in culture was found to be a limiting factor in its clinical application. Hence, we realized that alternate techniques to stimulate and propagate primed pre-effector cells needed to be identified. Since antigen recognition by T cells involves the TCR/CD3 complex for signal transduction and antibodies binding to the complex activate T cells, we examined whether *in* vitro antigenic stimulation could be achieved by using mAb to CD3 (8-10). This possibility was examined with several murine tumor models. A hamster mAb, 145-2C11, directed against the CD3ε chain of the murine TCR/CD3 complex has been characterized

(11). This antibody has a variety of in vivo and *in* vitro immunomodulatory effects on T cells including the induction of nonspecific proliferation. Tumor-draining LN cells activated for 2 days with anti-CD3 (1 ug/ml) alone did not result in significant proliferation. However, sequential activation with anti-CD3 followed by expansion in a low concentration of IL-2 (10 u/ml) for 3 days resulted in approximately 10-fold increase in cell numbers (8). This proliferation was not specific to the tumor-draining LN cells because normal LN cells proliferated to an equivalent degree. In spite of this, our adoptive immunotherapy experiments revealed that the anti-CD3 activated tumor-draining, but not normal, LN cells had antitumor effects.

Numerous reports have demonstrated that the in vitro stimulation of normal T cells with anti-CD3 results in proliferation and generation of cytotoxic effector cells (12,13). However, none of these anti-CD3 stimulated cells demonstrate antigen-specific reactivity probably due to the ubiquitous presence of CD3 on all T lymphocytes. Our experiments with anti-CD3/IL-2 activation of tumor-draining LN cells suggested that specific stimulation of antigen-sensitized pre-effector cells had occurred. We therefore examined the specificity of adoptive immunotherapy mediated by anti-CD3/IL-2 activated cells. Anti-CD3/IL-2 activated MCA 106 tumor-draining LN cells were transferred to mice with 3-day established metastases from three antigenically distinct murine sarcomas (Table 1). Mice lungs were harvested 14 days after tumor inoculation and metastases counted; too numerous to count tumors/lung were scored as > 250. There was significant antitumor reactivity against the MCA 106 tumor but not the MCA 102 or MCA 105 tumors. In separate experiments we found that similarly activated LN cells draining the MCA 105, MCA 203 or MCA 102 tumors mediated significant reductions of MCA 105, MCA 203 or MCA 102 metastases, respectively (data not shown) (11).

Table 1: Specificity of Adoptive Immunotherapy Mediated by Anti-CD3/IL-2 Activated MCA 106 Tumor-Draining Lymph Node Cells

Treatment		Mean no. pulmonary metastases (SEM)		
Anti-CD3 activated cells($2x10^7$)	IL-2 (15000 u, ip 2x/day for 4 days)	MCA 102	MCA 105	MCA 106
-	-	246 (4)	>250	>250
-	+	>250	>250	>250
+	+	217 (28)	>250	0*

*Compared to no treatment or IL-2 alone $p < 0.05$

Next, we examined the feasibility of generating anti-CD3/IL-2 activated effector cells reactive to the poorly immunogenic B16 BL6 melanoma. This subline of B16 melanoma, a spontaneously arisen tumor, is poorly immunogenic by virtue of the inability to elicit systemic immunity in syngeneic B6 mice experimentally. Additionally, therapeutically effective TIL cannot be generated from progressive subdermal tumors (B. Fox, data not shown). Initial experiments with the B16 BL6 tumor indicated that unlike the immunogenic MCA 106, therapeutic anti-CD3/IL-2 activated cells could not be induced from tumor-draining LN. However, it was possible to generate anti-CD3/IL-2 activated lymphocytes with therapeutic efficacy against the B16 BL6 tumor from LN draining an inoculum of tumor cells admixed with C. parvum (14,15). As noted in Table 2, the antitumor reactivity of tumor-primed anti-CD3/IL-2 activated LN cells was critically dependent upon the dose of bacterial adjuvant. Activated cells (5×10^7/mouse) were transferred i.v. into mice with 3-day established B16 BL6

pulmonary metastases. IL-2 (15,000 u i.p. bid x 4 days) was started on the day of adoptive transfer.

Table 2: Antitumor Efficacy of Anti-CD3/IL-2 Activated Cells Derived from B16 BL6 Draining LN is Dependent upon Amount of C. parvum.

Anti-CD3 activated cells	C. parvum (μgm)	Mean no. pulmonary metastases (SEM)
-	-	> 250
+	0	209 (38)
+	12.5	8 (6)*
+	25	< 1 (<1)*
+	50	187 (30)
+	100	173 (20)

$p < 0.05$ compared to group not receiving cells.

We also examined the kinetics of pre-effector cell induction after vaccine-priming. In this experiment, mice were inoculated with 10^6 viable or 10^7 irradiated (4,000 cGy) B16 BL6 tumor admixed with 12.5 μgm C. parvum s.c. every few days so that draining LN could be obtained on the same day for activation. The development of pre-effector cells in the draining LN was evident as early as day 4 after tumor priming but disappeared after day 14. These findings confirmed that irradiated B16BL6 tumor expressed appropriate tumor antigens and that the host immune response could be modulated to generate anti-CD3/IL-2 activated LN cells against this poorly immunogenic tumor. Based on these data, we are currently conducting a clinical study of similarly generated anti-CD3/IL-2 vaccine primed LN cells in patients with metastatic melanoma and renal cell cancer.

3.2 Clinical experience with human tumor-primed LN cells.

Extrapolating methods from our animal models, we initiated a clinical trial to evaluate the antitumor reactivity of tumor-primed IVS-LN cells (16). Patients with advanced melanoma or renal cell cancer were eligible. Patients were tumor-primed by the intradermal inoculation of 1-2 x 10^7 irradiated (2,500 cGy) autologous tumor admixed with fresh-frozen Tice BCG (10^7 cfu) in two separate sites. These sites were either on the anterior thigh or axilla in order that draining LN could be surgically removed under local anesthesia 10 days later for subsequent IVS culture. The use of BCG was employed since previous clinical studies demonstrated that it was an effective immune adjuvant in eliciting cellular immune responses when administered in conjunction with irradiated tumor (17,18). The yield of primed LN cells from 10 patients averaged 1.3 x 10^9 cells with removal of clinically hyperplastic LN. There were no complications associated with the tumor vaccination procedure nor removal of the primed LN.

Upon retrieval of primed LN, the lymphocytes were isolated and placed in IVS culture. IVS cultures were established in gas-permeable culture bags with complete media (CM) containing 1-2 x 10^5 LN cells/ml and 1-4 x 10^5 irradiated tumor cells/ml. CM consisted of RPMI 1640 with 10% human AB sera, 100 Cetus u/ml IL-2, antibiotics, L-glutamine, non-essential amino acids and sodium pyruvate. Culture bags were incubated at 37°C in 5% CO_2 for 10-15 days. Cells were harvested after tumor cells cleared from the culture and the

IVS lymphoid cells reached maximum density. IVS cells proliferated up to 14-fold during an average period of 12 days.

A mean of 6.7 x 10^9 cells were infused in 10 patients (7 melanoma, 3 renal cell cancer) along with the concomitant administration of IL-2 (180,000 IU/kg q8h for 5 days). Phenotype analysis of the IVS-LN cells revealed 78% T cells which were predominantly $CD4^+$. Seven of 9 patients that received IVS-LN cells developed delayed-type hypersensitivity (DTH) to autologous tumor suggestive that antitumor reactivity was passively transferred. Of the 10 patients treated with IVS-LN cells and IL-2, there was one partial and one minor response, and one patient remains with stable disease at 36+ months.

This initial study indicated that vaccine-primed LN cells harbored tumor reactive lymphocytes and that additional studies to generate larger numbers of cells might be required to evaluate their efficacy. A limiting factor for generating larger numbers of IVS cells was the amount of available tumor for the culture procedure. Therefore, we developed an alternate in vitro method to generate immune cells from tumor-primed pre-effector cells without the requirement of tumor stimulator cells (see Section 2.1). This alternative method utilized the stimulation of pre-effector cells by an anti-CD3 mAb for 48 hours followed by their expansion in IL-2 (10 u/ml). From our animal studies, this activation procedure resulted in the expansion and maturation of highly specific effector T cells.

Utilizing this approach we initiated a second clinical study (19). Patients were primed to autologous tumor utilizing the same vaccination procedure as was used for the IVS protocol. Tumor-primed LN cells were harvested 10 days after vaccination and activated by the anti-CD3/IL-2 method. Since it was anticipated that the primed LN would expand approximately 5 to 6-fold after the first activation procedure, further activation utilizing the identical procedure would be performed a second time. Animal studies in our laboratory have demonstrated that the antitumor efficacy of these activated cells were maintained during a second activation procedure. We anticipated that this would enable at least a 30-fold expansion of 10^9 primed LN cells which could be obtained from each patient. Not only would this represent a significantly larger number of immune cells generated for each patient, it would also increase the number of patients who could potentially be treated since large numbers of tumor cells were not required.

For anti-CD3 activation, lymphocytes were suspended in CM at a concentration of 2 x 10^6 cells/ml in flasks coated with immobilized anti-CD3 mAb (OKT3). After 2 days of activation the cells were harvested, washed and expanded in IL-2. Expansion in IL-2 was accomplished by resuspending 3 x 10^5 anti-CD3 activated cells/ml in fresh CM containing 10 u/ml of IL-2. Cells were grown to maximum density and then re-cultured in a secondary anti-CD3/IL-2 activation procedure. This activation scheme is illustrated in Appendix A with a patient who had 5 x 10^8 LN cells activated in vitro over a 13-day period to a final cell number of 1.4 x 10^{11} effector cells.

To date 14 patients (9 melanoma and 5 renal cell) have been treated on this protocol. A median of 2.8 x 10^{10} activated cells were administered in conjunction with IL-2 (360,000 IU/kg q8 x 5d). Phenotype analysis of the anti-CD3/IL-2 activated LN cells revealed that $CD8^+$ T cells were preferentially activated. Among the 9 patients with melanoma, 1 had a partial and 2 minor responses to treatment. Of note, among the 5 patients with renal cell cancer, 1 had a complete and 3 partial responses to therapy. Among the 5 patients with complete or partial responses, 3 developed DTH reactivity to autologous tumor. By contrast, among the remaining 9 patients, only 1 developed DTH reactivity to autologous tumor.

Currently, we are further pursing this clinical protocol in patients diagnosed with renal cell cancer. Our initial impression is that additional methods may be required to generate therapeutic activated T cells for the treatment of melanoma. To address this problem, we have explored the use of gene-modified tumor cells to upregulate the sensitization of vaccine primed LN cells. These observations are summarized in the next section.

3.3 Rationale for proposed clinical trial to utilize IL-4 gene modified tumor cells to prime LN in vivo.

Conceptually, the modulation of tumor immunogenicity by genetic manipulation can be categorized into two categories: 1) the expression of novel immunogenic antigens on the cell surface which will stimulate the host immune response (ie., allogeneic MHC antigens, B7, etc.), or 2) the elaboration of a gene product by the tumor cell which will facilitate the host immune response in a paracrine fashion. Supporting the latter, several recent studies have demonstrated that the transduction of murine tumor cells with genes encoding cytokines alter the tumorigenicity of the cells as well as upregulate the T cell response by the hosts inoculated with the tumors. Fearon et al. reported that the transduction of the murine tumor, CT26, with a gene coding for IL-2 resulted in the spontaneous regression of these tumors in the syngeneic host (20). After regression, these animals were found to have cytolytic T cells in their spleens reactive to parental tumor and developed systemic immunity documented by the rejection of a subsequent challenge of the parental tumor. Many other cytokines (ie, GM-CSF, IFNγ, TNFα, IL-4) examined in different murine tumor models have demonstrated similar findings. Golumbek et al. found that mice inoculated with the Renca renal cell tumor genetically engineered to secrete IL-4 were rejected in a T cell-independent manner (21). More importantly, these animals developed T cell-dependent systemic immunity to the parental tumor. Tepper et al. examined five other distinct tumor types and confirmed that genetic modification of these tumors to secrete IL-4 would lead to decreased tumorigenicity; and that this phenomenon was directly correlated to the amount of IL-4 secretion (22). In the latter study, the local infiltration of eosinophils was found to be a dependent factor in regression of the transduced tumors. Despite the potent antitumor response observed locally with cytokine transfected tumors, these and other studies have failed to document the capability of inducing a sufficient host immune response to mediate the regression of established disseminated tumors.

We have further examined the immunobiologic effects of IL-4 transduced tumors utilizing the poorly immunogenic B16BL6 murine melanoma. The B16BL6 tumor was cloned by limiting dilution technique and a clone, A9, was isolated. The A9 clone was transfected by electroporation with a murine IL-4 cDNA in the PLJ expression vector containing the neomycin-resistance gene (23). Transfectants were selected in G418 and clones were assayed for their ability to secrete IL-4. A clone, A94-3, was isolated and found to secrete 38 u/ml (2 x 10^5 cells/ml/24 hr). The in vivo tumorigenicity of A94-3 was compared to a control tumor clone which was transfected with the expression vector only (A9PLJ1). Syngeneic B6 mice (5/group) were inoculated with 10^6 tumor cells s.c. and tumor growth serially measured (see Figure, Appendix B). A94-3 tumor cells were found to have decreased tumorigenicity compared to A9PLJ1 tumors. Previous studies had documented that the tumorigenicity of A9PLJ1 was identical to unmodified parental A9 tumor cells (23).

The ability to establish pulmonary metastases after i.v. inoculation of A94-3 was also impaired compared to parental A9 tumor. B6 mice were inoculated with 2 x 10^5 tumor cells (5/group) and lung metastases enumerated 2 weeks later. The mean number of lung metastases $\pm$ SEM for the A9 and A94-3 groups was 185 $\pm$ 15 and 51$\pm$ 6, respectively.

These studies confirmed that IL-4 secretion by A9 tumor was associated with reduced tumorigenicity. However, none of the animals were found to be tumor free.

We proceeded to examine if A94-3 growth would elicit pre-effector cell sensitization in tumor-draining lymph nodes (TDLN). Mice were inoculated with 10^6 A94-3 or A9PLJ1 s.c. and 9-10 days later TDLN harvested to assess for pre-effector cell sensitization against parental tumor. TDLN were activated by the anti-CD3/IL-2 procedure we have previously described (see Section 2.1) and their antitumor reactivity assessed by the adoptive immunotherapy of established 3-day parental (A9) pulmonary metastases (Table 3). In these series of experiments, 4-5 x 10^7 cells were transferred with the concomitant administration of low doses of IL-2 (15,000 u i.p. bid x 4d). We found that LN draining A94-3 tumors mediated significant regression of A9 lung tumors compared to groups of mice which were not treated with activated cells. There was no antitumor reactivity of activated LN cells derived from A9PLJ1 bearing animals at the cell dose used.

Table 3: Adoptive Immunotherapy of Pulmonary Metastases with LN Cells Draining IL-4 Transfected B16-BL6

Source of LN Cells	Cells Transferred	Mean # Metastases (SE) Exp 1	Exp 2	Exp 3
	-	241 (18)	224 (26)	> 250
A9PLJ1	+	217 (28)	159 (28)	224 (21)
A94-3	+	76 (9)+	100 (35)	125 (29)+

*All mice received IL-2 i.p. (15,000 u bid) for 4 days. Cell dose was 4-5 x 10^7 per mouse.
+Significant difference with no treatment and PLJ1 control groups ($p < 0.05$).

We next examined the antitumor effect of these activated LN cells at larger doses. Utilizing the same protocol as Table 3, 7-13 x 10^7 activated cells were transferred into mice with established A9 parental lung metastases. As can be seen in Table 4, anti-CD3/IL-2 activated cells derived from A94-3 draining LN resulted in >97% reduction of A9 pulmonary metastases in both experiments (Appendix C).

Table 4: Immunotherapy of 3-day BL6 (A9) Pulmonary Metastases with Activated TDLN Cells From Mice Bearing IL-4 Transfected Tumor (A94-3)

Source of TDLN	Cells Transferred[a]	Mean No. of Pulmonary Metastases (SEM) Exp 1	Exp 2
-	-	>250	> 250
A9	+	97 (16)[b]	n.d.
A94-3	+	12 (6)[c]	0[b]

[a]No. of cells: Exp. 1 (7 x 10^7) and Exp. 2 (1.3 x 10^8). All mice received 15,000 u IL-2 i.p. bid for 5 days after cell transfer.
[b]$p < 0.05$ compared to no cell treatment.
[c]$p < 0.05$ compared to all other groups.

From our previous animal studies, we had documented that the bacterial adjuvant, C. parvum, was useful in upregulating the in vivo sensitization of TDLN pre-effector cells against poorly immunogenic tumors (see Section 2.1). Utilizing this model, we compared the effect of C. parvum in eliciting sensitized pre-effector cells to A9 tumor compared with A94-3. Groups of B6 mice were inoculated s.c. with: A9PLJ1 tumor ± C. parvum or A94-3 tumor ± C. parvum.

Ten days later TDLN were harvested and activated by the anti-CD3/IL-2 method. Antitumor reactivity was assessed by the adoptive immunotherapy of A9 pulmonary metastases as previously described. As shown in Table 5, A9PLJ1 did not elicit therapeutic TDLN, however, the use of C. parvum plus A9PLJ1 resulted in activated TDLN with antitumor efficacy confirming our previous findings. In comparison, activated TDLN from mice inoculated with A94-3 alone had comparable antitumor reactivity compared to cells from mice inoculated with A9PLJ1 plus C. parvum. In the same experiment, we examined if there was an additive effect of C. parvum with A94-3 cells in the stimulation of pre-effector TDLN cells. The addition of C. parvum to A94-3 did not enhance nor abrogate the antitumor reactivity of the activated TDLN cells in adoptive immunotherapy.

Table 5: Immunotherapy of 3-Day BL6 Lung Metastases with Activated TDLN Cells: Effect of C. parvum as an Adjuvant

Source of TDLN		Cells	Mean Pulmonary
Tumor	C. parvum[a]	Transferred[b]	Metastases (SEM)
-	-	-	> 250
A9PLJ1	-	+	224 (21)
A9PLJ1	+	+	115 (21)[c]
A94-3	-	+	125 (29)[c]
A94-3	+	+	123 (35)

[a]C. parvum (13 mcg) admixed with 10^6 tumor cells followed by TDLN harvest 10 days later.
[b] 5×10^7 cells were transferred per mouse. All mice received 15,000 u IL-2 i.p. bid for 4 days after cell transfer.
[c]$p < 0.05$ compared to no cells and A9PLJ1 control groups.

Our group has already established considerable experience in utilizing autologous tumor cells plus the bacterial adjuvant, BCG, to stimulate LN cells in cancer patients for subsequent adoptive immunotherapy. The preliminary results of this experience appears to be very encouraging for patients with renal cell cancer; however, the need to develop improved methods to treat melanoma is apparent. The studies summarized in this section indicate that IL-4 transfection of the B16BL6 melanoma can upregulate T cell sensitization in draining LN. These draining LN can be further activated in vitro to successfully eradicate established disseminated parental tumors. We therefore propose in this study to vaccinate patients with autologous tumor cells which are transduced with the IL-4 gene. Upon documentation of IL-4 production by the tumor cells, they will be irradiated and inoculated i.d. into patients near LN bearing areas. Approximately 7 to 10 days later, draining LN will be removed for anti-CD3/IL-2 activation. The activated cells will be subsequently infused i.v. into patients followed by the concomitant administration of IL-2 (360,000 IU/kg q8h for 5 days).

4.0 DRUG INFORMATION

4.1 Interleukin-2

The recombinant IL-2 (Proleukin^R) used in this protocol is manufactured by Cetus Corporation, Emeryville, CA and will be supplied by the Cancer Treatment Evaluation Program (CTEP), NCI. Proleukin^R is a protein manufactured by recombinant technology and is similar to the native IL-2 protein found in humans. Proleukin^R is supplied as a lyophilized cake in 5 ml vials containing 1 mg of protein. Each vial should be reconstituted with 1.2 ml of sterile water and gently swirled without excess foaming. When reconstituted each ml contains 1 mg (18 million IU) of Proleukin^R. For intravenous injection, Proleukin^R can be

reconstituted in volumes of 50 to 500 ml of 5% dextrose solution with 0.1% human albumin. It is recommended that in-line filters not be used for delivery of Proleukin^R since bioassays have shown significant loss of IL-2 activity after filtration. Reconstitution with sterile water or normal saline solutions for intravenous injection should be avoided because of precipitation. In addition, Proleukin^R should not be mixed with other drugs. The lyophilized material is stable at 2-8°C for 18 months from the date of manufacture. This material should be protected from excessive exposure to light during extended storage.

4.2 OKT 3

OKT 3 is a murine monoclonal antibody (mAb) to the CD3 antigen on human T cells. It is manufactured by Ortho Pharmaceutical Corp. and will be supplied by CTEP, NCI. It has been used extensively as an intravenously administered immunosuppressive agent in organ transplant patients. The mAb is a biochemically purified IgG_{2a} immunoglobulin. It is supplied in a 5 ml ampule (Ortho clone OKT 3^R) containing 5 mg (1 mg/ml) of mAb in a sterile, colorless solution which may contain a few fine translucent protein particles. Each ampule contains a buffered solution (pH 7.0 ± 0.5) of monobasic sodium phosphate (2.25 mg), dibasic sodium phosphate (9.0 mg), sodium chloride (43 mg) and polysorbate 80 (1.0 mg) in water.

4.3 Retroviral vector: GBAH4-8

GBAH4-18 is a Moloney Murine Leukemia virus vector which encodes for human IL-4. The retrovirus producer cell line is currently undergoing safety testing as is required by the FDA. ***Once testing is complete, then a 1-2 liter lot of virus stock will be prepared by an FDA-approved commercial facility and subjected to tests for replication competent virus, bacterial, viral, and fungal agents as is required clinical grade material produced by cell lines.***

4.3.a. Construction of the retrovirus vector GBAH4-18.

The parental retrovirus vector used for these studies is the gag BA, which has been extensively studied, and was the parental vector for a previous human gene therapy protocol approved by the RAC (#9110-12). The general structure of the vector will be described, using the published numbering of the provirus sequences (24). The backbone structure of this vector includes an intact 5' LTR of Moloney Murine Leukemia Virus (Mo-MLV), with additional Mo-MLV sequences between the 5' LTR and the internal promoter spanning nucleotide 146 at the border of U5 to the natural Xho 1 site in the gag coding region at nucleotide 1560 (with the exception that a Sac II linker was inserted at the Hae III site at nucleotide 624 (25). The plasmid also contains wild-type Mo-MLV sequences from the Cla I site at nucleotide 7674 (which was converted to a Bam H1 site with synthetic linkers) to the end of the 3' LTR. Sequences containing the viral enhancer elements of the 3' LTR from the Pvu II site at nucleotide 7933 to the Xba site at nucleotide 8111 have been deleted. In addition to these sequences, there are flanking mouse genomic DNA and pBR322 sequences (spanning the Hind III site to the Eco R 1 site). The internal promoter used in this vector was derived from a Xho 1 to Mbo 1 fragment of the chicken β-actin gene spanning nucleotides -266 to +1(26). The Mbo 1 site was converted to a Bam H1 site and the modified β-actin fragment was cloned into the parental vector. The plasmid pCDhIL-4, containing the complete human IL-4 cDNA, was digested with Mse 1, and a 472 base pair fragment containing base pairs 61-513 of the human IL-4 cDNA sequence was obtained (27). The fragment was blunt ended with Klenow and deoxynucleotides, synthetic Bam H1 linkers

added, Bam H1 digested, and cloned into the plasmid pMFG (28). The plasmid pMFG was Bam H1 digested, the 470 base pair fragment containing the IL-4 cDNA was isolated, and cloned into the retrovirus backbone gagBA. The orientation of the IL-4 sequence, and the integrity of the plasmid provirus DNA structure were confirmed by restriction endonuclease digestion. A schematic of the plasmid map is shown in Appendix D, followed by the restriction map of the provirus and the anticipated complete sequence of the provirus. The plasmid provirus vector is currently being completely sequenced to confirm that no inadvertent mutations have occurred during plasmid DNA subcloning.

4.3.b. Generation of recombinant retroviruses encoding for human IL-4

Virus producing cell lines were isolated for the vector using the amphotropic packaging cell line Ψ_{crip}, kindly provided by Dr. James Wilson (29). The Ψ_{crip} cell line was constructed by transfecting the gag-pol and env functions of the retrovirus on separate plasmid constructs into NIH3T3 cells. The 3' LTR's of the constructs were replaced by heterologous polyadenylation signals. These modifications were performed to minimize the chance that recombination could result in the production of replication competent virus. The plasmids used to make the packaging cell line were described in the original paper.

The retrovirus vector GBAH4 was cotransfected into Ψ_{crip} with pSV2Neo, and stably transfected clones were selected in 1 mg/mL G418. The producer cell lines are maintained in Dulbecco's modified Eagle's media supplemented with 10% calf serum and penicillin/streptomycin. After screening 22 virus producing clones, the best clone (GBAH4-18) was further characterized. Virus supernatant was used to transduce exponentially growing NIH3T3 cells and human melanoma cells. IL-4 secretion after transduction with GBAH4-18 ranged from 100-2000 pg/ml (Appendix E). Southern blot analysis was performed with cells transduced with the GBAH4-18 virus (Appendix F). This revealed approximately 1 copy of unrearranged virus in the NIH3T3 cells. Optimization of the transduction protocol with melanoma cell lines is continuing to attempt to improve this transduction efficiency. Virus supernatant has undergone a helper assay with 15 days of 3T3 amplification. This showed no evidence of helper virus.

The virus producer cell line is currently undergoing further safety testing as required by the FDA of clinical grade retrovirus stocks. This includes testing for replication competent virus, for the presence of bacterial, viral and fungal pathogens. The retrovirus supernatant will be produced by a commercial supplier and will not be used to transduce melanoma cells for human trials until approval is received from the FDA.

5.0 ELIGIBILITY CRITERIA

5.1 Eligible patients will include those with the following (see Appendix G):

a. Histologically proven metastatic melanoma.
b. Performance status ECOG 0-1 (see Appendix H).
c. No symptomatic or acutely life-threatening tumor which is judged likely to require intervention with alternative modalities for 3 months.
d. Non-tumor involved superficial inguinal or axillary lymph nodes which are surgically accessible.
e. Absolute WBC >3000/mm^3.
f. BUN < 25 mg, creatinine $\leq$ 1.8 mg%

g. Liver function tests: total bilirubin ≤2.0 mg.dl, SGOT ≤70 IU/L, SGPT ≤90 IU/L.
h. 18 years or older

5.2 Patients will be excluded for:

a. Prior second malignancy (except squamous or basal cell skin cancer)
b. Previous severe reactions to any blood product.
c. Chronic renal disease with creatinine ≥ 2X normal
d. Previous chemotherapy, radiation therapy, or steroids within 4 weeks.
e. Brain metastasis.
f. Active infection requiring treatment or unexplained febrile illness, any collagen disease or autoimmune process.
g. History of ischemic or congestive cardiac disease requiring chronic medication (NYHA class III, IV) or evidence of ischemic change or venticular ectopy (>4/min) on electrocardiogram, or evidence of type II AV block. Any evidence of prior or current cardiac disease as determined by stress test and EKG.
h. Functional respiratory impairment (FEV_1 < 2.0 liters or < 75% predicted)
i. Pregnancy, or men and women of procreation potential who refuse to take precautions to avoid pregnancy.
j. Patients who are HIV positive.

6.0 TREATMENT PLAN

Patients with advanced melanoma will undergo surgical retrieval of tumor for use as a tumor vaccine. A tumor cell line will be established in culture and subsequently transduced with a retroviral vector containing the IL-4 gene. The transduced line will be screened for IL-4 production and subsequently irradiated with 5,000 cGy. Patients will be vaccinated with 10^7-10^8 irradiated transduced tumor cells intradermally in 1 or 2 sites. Surgical removal of draining LN 7 to 10 days after vaccination will be performed to obtain lymphocytes for in vitro activation with anti-CD3 monoclonal antibody (OKT3) and expansion in IL-2. After in vitro activation, these lymphocytes will be adoptively transferred intravenously to the patient followed by the administration of IL-2 (360,000 IU i.v. every 8 h. for 5 days). Fifteen patients will be treated to gain sufficient information regarding toxicity, antitumor reactivity and immunologic function. Retreatment will be considered if the patient has a response and cryopreserved LN cells are available. This retreatment will be performed 2 months after completion of the last therapy and will involve repeat infusion of activated lymphocytes and IL-2. ***The procedures for tumor preparation, LN cell preparation and anti-CD3/IL-2 activation have been previously reviewed and approved by the FDA (IND 3860) for prior clinical studies.***

6.1 Preparation of Single Cell Suspensions and Cultures from Solid Tumors

Resected tumors will be collected at surgery and transported in cold (4°C) RPMI 1640 media (GIBCO) until processing. Necrotic tumor and connective tissue will be trimmed and the remaining specimen will be minced to approximately 3-4 mm^3 pieces with scissors in HBSS containing 2.5 units/ml hyaluronidase, 0.5 mg/ml collagenase and 0.05 mg/ml deoxyribonuclease. All enzymes are obtained from Sigma Chemical Co., St. Louis, MO in lyophilized form. The enzymatic digestion of solid tumors will be carried out at room temperature for 3-5 hours with constant stirring in sterile trypsinizing flasks containing magnetic stirring bars. The stirring plate will be set between 3-4 to allow turbulence without

foaming. Routinely one gram of tumor specimen will be digested in a minimum volume of 10 ml enzyme mixture. The resulting cell suspension will be centrifuged (500 g, 15 min.), filtered through a double layer of no. 100 nylon mesh and resuspended in 100 ml RPMI 1640 medium containing 10% heat-inactivated human AB serum. If there are significant residual tumor pieces remaining in the trypsinizing flask they will be digested by repeating the above protocol. An aliquot of harvested tumor cells will be sent for cytologic analysis and for bacteriological testing. The remaining tumor cells will be cryopreserved in 90% human AB serum plus 10% dimethylsulfoxide at -178°C in liquid nitrogen for patient skin testing and in vitro assays. Each freezing vial will contain 2 to 4 x 10^7 tumor cells. The procedures for tumor procurement and preparation will be carried out by sterile techniques.

In addition to cryopreservation, a tumor cell line will be initiated by suspending approximately 60 ml of complete media (CM) in 150 cm^2 flasks with 1-5 x 10^6 freshly dissociated tumor cells/flask. CM is composed of RPMI 1640 with 10% human AB serum, 2 mM glutamine and 50 µg/ml gentamicin. Flasks are incubated at 37°C in 5° CO_2. It is anticipated that only 3-4 gm of tumor will be required from each patient for therapy.

6.2 Transduction of melanoma cells with the IL-4 encoding retrovirus vector.

All procedures for tumor transduction will be performed in the Human Applications Laboratory of the CRC. This is a BL-2 facility dedicated for human gene therapy applications. FDA certified, frozen virus supernatant will be thawed in preparation for melanoma cell transduction. The thawed virus will be filtered through a 0.22 u filter, supplemented with protamine (5 ug/mL) and placed on cultured melanoma cells. The virus will be exposed to the cultured melanoma cells for a 16 hour period. The virus will be removed, and the cells will be placed in CM for an additional 24 hour period. The conditioned media will be harvested and assayed for IL-4 production. If IL-4 production is of high enough quantity to prepare a tumor cell vaccine (ie, greater than 50 pg/mL/10^6 cells/24 hours), the cells will be washed, harvested, and used for vaccine preparation as described below.

6.3 Vaccination with IL-4 modified tumor cells

Gene-modified tumor cells will be harvested and irradiated with 5,000 cGy. The cell suspension will be adjusted so that a volume of 0.5 ml HBSS contains 10^7-10^8 irradiated tumor cells. The tumor cells will be placed on ice until injection. If 10^8 cells are not available, fewer may be given but not less than 10^7 will be injected per site. Patients will be immunized i.d. with 0.5 ml of the cell suspension in one or two separate sites drained by an accessible nodal basin (ie. inguinal or axillary region). An india ink tatoo will be placed to mark each site of the injection. Lymph nodes draining the sites of vaccine will be selected so that the chosen LN group will not have clinically apparent metastases. Before immunization, patients will have been skin tested with a standard panel of microbial recall antigens (Merieux multi-test) as well as irradiated (10,000 cGy) irradiated autologous tumor cells in graded doses. These skin tests are performed to determine the immunocompetence of the patient as well as obtain pre-treatment evaluation of cutaneous reactivity to autologous tumor antigens.

6.4 Preparation of single cells from lymph nodes

Patients will undergo excision of hypertrophied LN draining the site 7 to 10 days after the vaccination. This can be performed under local anesthesia as an outpatient procedure. Lymph nodes will be transported to the laboratory in sterile containers containing HBSS. Single cell suspensions will be prepared mechanically by mincing with scissors to

approximately 2 mm^3 pieces in cold HBSS. Cells will be dissociated by pressing pieces of LN through a sterile steel mesh with the blunt end of a 10 ml syringe plunger. The resultant cell suspension will be filtered through a double layer of no. 100 nylon mesh. After washing the cells in HBSS x 3, they will be placed in anti-CD3 activation cultures immediately and the remaining LN cells will be cryopreserved in liquid nitrogen for subsequent analysis and possible use in retreatments. All procedures will be carried out under sterile conditions.

6.5 Anti-CD3 Activation and Expansion

Animal studies of adoptive immunotherapy have clearly demonstrated that the numbers of transferred effector cells are directly related to the antitumor effects which were observed. Clinical studies which have reported tumor responses from the adoptive transfer of LAK or TIL cells have generally administered 10^{10} to 10^{11} effector cells (1,2). From our current clinical studies, we have been able to generate these numbers of human activated LN cells utilizing the proposed anti-CD3/IL-2 activation procedure (see Section 2.2). This required culturing cells an average of 15 days and subjecting the draining LN cells to a primary and secondary culture procedures. The methods for the primary and secondary culture procedures utilized in this clinical study are described below:

6.5.a Primary Anti-CD3 Activation

For primary anti-CD3 activation, 5 x 10^8 lymphocytes will be suspended in 250 ml of complete media (CM) and cultured in 24-well tissue culture plates pre-treated with OKT3. Plates are coated with mAb by adding 400 µl of diluted OKT3 (1 µg/ml) in sterile 0.05 M borate buffer, pH 8.6 per well and stored overnight at 4°C. Prior to use, the plates are washed of excess mAb with PBS. CM is X-Vivo-15 (Whittaker) supplemented with 10% human AB serum (Sigma). After 48 hours of activation the cells are harvested and expanded in IL-2.

Expansion in IL-2 is accomplished by suspending 3 x 10^5 anti-CD3 activated cells/ml by diluting the primary activation media with X-Vivo-15 containing 5% human AB serum supplemented with 60 IU/ml of IL-2 (Cetus, Emeryville, CA). These cells are expanded in 3000 ml culture flasks (Lifecell 3000, Fenwal, Deerfield, IL) each containing 500-850 ml of media. When cell density reaches approximately 10^6 cells/ml, an additional 2 liters of serum-free X-Vivo-15 is added to each flask. Cells are grown to a maximum density (approximately 2 x 10^6 cells/ml) which is anticipated to take 5 days based on our current experience.

6.5.b Secondary Anti-CD3 Activation

The procedure to perform the secondary anti-CD3 activation represents a slight modification of the primary culture procedure. Briefly, 2 x 10^8 anti-CD3 activated cells derived from the primary culture procedure will be suspended in 100 ml of CM in 150 cm^2 flasks with immobilized OKT3 mAb. After 1 day, the cells are harvested, washed and expanded in serum-free media (X-Vivo-15) containing IL-2 (60 IU/ml) using 3000 ml culture bags as previously described. Cells are harvested after a 3-4 day period when cells reach their maximum density. It is anticipated that each patient will require 50-100 L of CM to generate anti-CD3 activated cells from primary and secondary culture

procedures. In order to handle large-scale tissue cultures, we plan to use automated systems for solution transfer and cell harvesting. A solution transfer pump, life cell tissue culture bags and the Celltrifuge II (Fenwall, Deerfield, IL) will be utilized.

6.5.c Harvesting Conditions

One out of ten bags will be tested for sterility 48 hrs. before harvesting by obtaining an aliquot of cells and sending it for culture by the Microbiology Laboratory of the University Hospital. Bacterial monitoring will include thioglycolate broth, chocolate agar and sheeps blood agar. Cell suspensions from the culture bags will be harvested and washed in phenol-free HBSS using the Celltrifuge II (Fenwal). A gram stain of the final cell pellet will be evaluated to confirm that there are no organisms prior to infusion.

6.6 Adoptive Cellular Therapy and IL-2 Administration

The lymphocytes will be suspended in infusion media consisting of 190 ml normal saline, 10 ml human albumin (concentration 25%, final concentration of 1.25%) and 450,000 IU IL-2. IL-2 will be administered i.v. to enhance the activity of the activated cells and will be given 3 times daily for five days starting on the day of cell transfer. Infusions of IL-2 will be 360,000 IU/kg and will commence immediately following cell transfer. This schedule and dose of IL-2 has been associated with manageable and reversible side effects according to our previous experience with this regimen. The patients will be treated as inpatients in the Clinical Research Center.

7.0 TOXICITIES TO BE MONITORED/DOSAGE MODIFICATIONS

7.1 Vaccination with retroviral transduced tumor cells

Inoculation with a retrovirus-transduced tumor cell vaccine could theoretically pose risks to the patient which include: a. Possible exposure to replication competent retrovirus, b. Possible adverse effects from provirus integration, c. Growth of the inoculated tumor at tumor vaccine site, d. Systemic toxicity from IL-4 production by the tumor cell vaccine, and e. Generation of autoimmunity by the tumor cell vaccine.

7.1.a Possible exposure to replication competent retrovirus.

Retrovirus transduction of tumors for vaccine preparation will only be performed with FDA approved retrovirus stocks in a dedicated facility (the Human Applications Lab) located in the Clinical Research Center at The University of Michigan. All retrovirus stocks will be safety tested and documented to be free of replication competent virus, bacterial, viral, and fungal adventitial agents before use in tumor transduction for clinical trials.

7.1.b Possible adverse effects from provirus insertion.

The tumor cells will be irradiated prior to administration to patients. This should provide for the complete loss of ability of the tumor cells to divide and or persist in the patients. Thus no growth of the tumor cells is expected, and therefore any possible mutagenesis from provirus integration should not result in harmful effects. If any tumors do

grow at the inoculation site, they will be biopsied, and assayed for IL-4 production, and for abundance of provirus sequences.

7.1.c Growth of the inoculated tumor at tumor vaccine site.

These patients will receive up to 10^8/irradiated transduced tumor cells intradermally in one or two sites. Tumor cells will be irradiated with 5,000 cGy. In our previous experience, inoculations of 2 x 10^7 irradiated (2,500 cGy) tumor cells admixed with BCG has not resulted in tumor outgrowth in more than 20 patients. Berd et al. has also reported an extensive experience in 64 melanoma patients treated with multiple injections of 1-2.5 x 10^7 irradiated (2,500 cGy) autologous tumor cells plus BCG without evidence of tumor outgrowth at the injection sites (17). The vaccine sites will be carefully monitored, and if tumor growth does occur the sites have been selected so that they can easily be excised with minimal morbidity. A small chance does exist, however, that if this site does grow that it might lead to the spread of injected tumor cells to other sites of the body. This unlikely event should not negatively influence the prognosis of this patient population which already has documented metastatic disease with limited life expectancy.

7.1.d Systemic toxicity from IL-4 production by the tumor cell vaccine.

If gene modified tumor cells grow and produce IL-4, then patients may be exposed to systemic IL-4. In theory, if a palpable tumor nodule were evident at 1 cm in size, approximately 10^9 cells would be present and secreting at least 50 ng of IL-4/24 hrs (minimum required level of IL-4 secretion is 50 pg/10^6 cells/24 hr). The maximum tolerable dose of intravenous bolus IL-4 per day has been reported to be 45 mcg/kg/24 hr (30). In a 50 kg patient, the amount of IL-4 potentially secreted by 10^9 cells would represent less than 0.01% of the maximum tolerable dose. We will monitor for any IL-4 toxicity by measurement of serum IL-4 levels.

7.1.e Generation of autoimmunity by the tumor cell vaccine.

If IL-4 production within an autologous tumor unlocks immunity to several self antigens, it may predispose the patient to develop autoimmunity and/or an autoimmune like syndrome. Tumor vaccines have been used throughout this century without such an effect, and in our BCG/autologous cell trial we have not seen evidence of clinical autoimmunity. We will measure anti-DNA antibodies to monitor for this possibility.

7.2 Cellular Therapy/IL-2 Administration

In all clinical studies of adoptive immunotherapy the major toxicities have been generally attributable to the infusion of high-dose IL-2 (ie. 720,000 IU/kg i.v. q8h). At this dose, the most prominent side effects are hypotension, leaky capillary syndrome, fluid retention, oliguria, respiratory distress and mental confusion (1,2). Since the side effects are dose related, it is anticipated that the toxicity associated with this proposal should be less since the dose of IL-2 being administered is 360,000 IU/kg i.v. q8h for a total of 5 days. With our current clinical studies, we have treated 14 patients with activated vaccine-primed LN cells and IL-2 at the proposed dosage without life-threatening toxicities.

Medications are used to overcome some of the side effects of treatment. Fever, chills and malaise are significantly reduced by the use of acetaminophen (650 mg every six hours) and indomethacin (25 mg every six hours). Many patients receive hydroxyzine hydrochloride, an antihistamine, for treatment of a generalized erythematous rash. Patients

are also maintained on ranitidine (150 mg orally bid) for prophylaxis against gastrointestinal bleeding. Patients receiving anti-hypertensive medications will have them discontinued during treatment with IL-2.

Toxicities of treatment will be recorded daily during treatment and graded on a 0-4 scale as defined by the NCI Common Toxicity Criteria (0 = no toxicity, 4 = life threatening, see Appendix I). For grade 4 toxicity, treatment will be discontinued. Based on our clinical experience and the experience reported by other groups. The dose modifications related to individual organ toxicity will be followed as indicated in Appendix J. Doses of IL-2 which are held will be restarted if the specific toxicity resolves to the next acceptable lower level.

8.0 STUDY PARAMETERS

8.1 Pretreatment patient evaluation

a. Complete history and physical examination
b. Chem-21 blood chemistry panel
c. CBC, differential, PT, PTT, platelet count
d. Urinalysis
e. HB_sAg, HTLV III Antigen
f. Anti-DNA antibodies
g. Chest CT scan
h. Abdominal CT scan
i. Head CT scan
j. Bone scan
k. Pregnancy test (if woman of child-bearing age)
l. Stress myocardial function tests if clinically indicated
m. PFT's if clinically indicated

8.2 Adoptive cellular therapy/IL-2 administration

Daily CBC, differential, platelet count, Chem-21

8.3 Post-treatment patient evaluation

a. History and physical examination with chest x-ray, Chem 21, and CBC every month post-treatment until responsive status is determined.

b. Chest and abdominal CT scan as appropriate to follow evaluable disease monthly for 2 months.

8.4 Immunologic studies: will be performed pre and post treatment (1 and 2 months after therapy)

a. Recall skin tests: Merieux multi-test

b. Cutaneous hypersensitivity to tumor: i.d. 10^4, 10^5 and 10^6 irradiated tumor cells (10,000 cGy) in the contralateral extremity

8.5 Assessment for adverse effects due to the retroviral transduced tumor cells

Serum IL-4 assays will be performed prior to tumor vaccination, at the time of LN harvest, at the time of adoptive cellular therapy; and at 1 and 2 months after completion of therapy.

9.0 CRITERIA FOR EVALUATION/REMOVAL FROM STUDY/RETREATMENT

Definition of responses: to be determined after a minimum of 4 weeks.

9.1 Complete tumor response (CR) is defined as disappearance of all signs, symptoms, biochemical, and radiographic evidence of tumor. In the case of accessible cutaneous or subcutaneous metastases, tissue biopsy of at least one known site of tumor is requested.

9.2 Partial response (PR) is defined as a reduction of all measurable tumor lesions by 50% of the product of the two greatest perpendicular diameters (sum of all evaluable tumors), without the appearance of new tumor lesions or the concurrent progression of any previously defined lesions.

9.3 Minimal response (MR) is defined as a reduction of all measurable tumor lesions by $\geq$ 25% but < 50% of the product of the two greatest perpendicular diameters (sum of all evaluable tumors), without the appearance of new lesions or the concurrent progression at any sites of known disease.

9.4 Stable disease (SD) includes all with response less than described for progressive disease.

9.5 Progressive disease (PD) is defined as an increase of all measurable tumor lesions by $\geq$ 25% of the product of the two greatest perpendicular diameters (sum of all evaluable tumors), or the appearance of new lesions.

9.6 Patients that recur at any site will be considered as failures of that treatment and other standard or experimental treatments will be considered as appropriate for their disease. If a tumor response (PR or CR) is evident by 2 months post treatment, then retreatment with activated cells from cryopreserved material during the following month will be offered to the patent. If cryopreserved LN are not available, then retreatment with IL-2 alone will be offerred.

10.0 STATISTICAL CONSIDERATIONS

Fifteen patients will be enrolled into this study. This has been deemed adequate to determine several in vitro and in vivo immunologic parameters which are being followed. Cytotoxicity, proliferation and cytokine release (ie. TNF or IFNγ) to autologous tumor will be measured of the vaccine-primed LN in vitro. Evidence of tumor-specific responses in these assays would be a basis for a phase II study since an antitumor effect may be related to the quantitative requirement of larger numbers of adoptively transferred cells. Similarly, if there is evidence for the development of cutaneous hypersensitivity to autologous tumor after adoptive immunotherapy in the majority of patients, then a phase II study is justified. Any objective tumor response in this study (PR or CR) will justify a phase II study.

11.0 ETHICAL & REGULATORY CONSIDERATIONS

The following will be observed for compliance with Food and Drug Administration regulations involving the conduct and monitoring of clinical investigations; they also represent sound research practice:

11.1 Institutional Review

This study will be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56). The protocol and informed consent form for this study will be approved in writing by the appropriate Institutional Review Board (IRB). The IRB will be from an institution which has a valid Multiple Project Assurance, Single Project Assurance or Cooperative Oncology Group Assurance on file with the Office for Protection from Research Risks, National Institutes of Health. The institution must be in compliance with regulations of the Food and Drug Administration and the Department of Health and Human Services.

Significant changes to the protocol, as well as a change of principal investigator, will also be approved by the Board and documentation of this approval provided to the study monitor. Records of the Institutional Review Board review and approval of all documents pertaining to this study will be kept on file by the investigator and are subject to FDA inspection at any time during the study. Periodic status reports will be submitted to the Institutional Review Board at least yearly, as well as notification of completion of the study and a final report within 3 months of study completion or termination. The investigator will maintain an accurate and complete record of all submissions made to the Institutional Review Board, including a list of all reports and documents submitted.

11.2 Drug Accountability

For each drug supplied for this study an accountability ledger containing current and accurate inventory records covering receipt, dispensing, and the return of study drug supplies will be maintained. The ledger will be maintained routinely for all studies regardless of study design, and will identify for each shipment the subject number (as applicable) and the quantity of drugs contained in the shipment. The ledger will consist of drug Accountability Record Forms supplied by the NCI. One form for each investigational drug used on each research protocol will be kept. A separate drug Accountability Form will also be maintained for each different strength or dosage form of the particular drug being used.

Drug supplies will be kept in a secure, limited access storage area under the recommended storage conditions. During the course of the study, the following information will be noted on the accountability ledger; the identification code of the subject to whom drug is dispensed, the date(s) and quantity of drug dispensed to the subject, and the date(s) and quantity of drug returned by the subject; subjects should return empty containers to the investigator, and the return noted on the ledger. These Accountability Forms will be readily available for inspection and are open to FDA inspection at any time.

11.3 Reporting "ADR" (Adverse Drug Reactions)(NCI 12/89)

The Federal (NCI and FDA) guidelines and conditions under which an "ADR" should be reported are given in Appendix K. The appropriate forms will be completed and submitted to both the NCI and to the University IRB Committee. This reporting will be performed according to the guidelines listed below.

11.4 University of Michigan Cancer Center Reporting Requirements of "ADR"

In addition to notifying the appropriate Federal and Group or Drug Company monitors, the University of Michigan Medical Center and the VA Medical Center both have internal notification procedures to follow. A copy of the completed form will be sent to the following groups: Nursing, Pharmacy, IRB Committee and appropriate medical staff.

If the "ADR" is an unknown reaction which has not been previously reported for the drug in question, the consent form will also be modified to include the previously-unknown toxicity. If this is necessary, the modified consent will be submitted immediately to the IRB Committee.

If the protocol in question is sponsored by the Cancer Center or one of the programs within the Cancer Center, the Protocol/Data Management section of the Clinical Trials Office will carry out the appropriate procedures for the "ADR" notification to both federal and hospital departments.

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The University of Michigan Medical School
and the Clinical Research Center

CONSENT FORM

(To be read by the Patient and explained to the
Patient by his or her Physician).

PROTOCOL: Adoptive Immunotherapy of Melanoma with Activated Lymph Node Cells Primed In Vivo with Autologous Tumor Cells Transduced with the IL-4 Gene

PRINCIPAL INVESTIGATOR: Alfred E. Chang, M.D.

CO-PRINCIPAL INVESTIGATORS: John C. Krauss, M.D.
Scott E. Strome, M.D.
Suyu Shu, Ph.D.

PROTOCOL NUMBER:_____ DATE: _____

PATIENT NAME: _____ HOSPITAL NO.: _____

INTRODUCTION

I am being invited to participate in a research study at the University of Michigan Medical Center. I understand that several general principles apply to all who take part in any experimental studies:

1. My participation in this study is voluntary.
2. I may not personally benefit from this study, but knowledge may be gained from it that will benefit others.
3. I may withdraw from the study at any time for any reason without jeopardizing my further care.

I am aware of the nature of the study, the risks, inconveniences, discomforts, and other information which are discussed in the following sections.

DESCRIPTION OF TREATMENT

I have a cancer which cannot be cured by medicine, surgery or radiation. In this study, treatments will be offered that may help to fight this disease in future patients. Because the treatment is experimental, I may not derive any direct benefit from it. I understand that the purpose of this study is to determine the safety of this new treatment regimen which will attempt to cause tumor shrinkage.

I will have removal of a sample of my own tumor which will be subsequently processed in a laboratory. The tumor will be modified in the laboratory by inserting a gene into the cells which will cause them to make a protein called interleukin-4 (IL-4). IL-4 is an immune hormone normally produced by the body. After this laboratory manipulation which will take 2 or 3 weeks, I will be injected with IL-4-modified tumor cells in one or two skin sites of my thigh or underarm areas. These modified tumor cells will be irradiated prior to injection into the skin in order to prevent their growth. About 7 to 10 days later I will undergo removal of lymph nodes that are adjacent to these injected skin sites in order to grow cells called lymphocytes that will later be given back to me as part of my treatment. The lymphocytes will be returned to me through an intravenous line placed in a blood vessel. In addition to these lymphocytes, I will receive interleukin-2 (IL-2), a protein normally made by the body's immune system that can help the immune cells function in my body.

PROCEDURES TO BE UNDERTAKEN

Before receiving any treatments, I will have a number of tests to determine if I qualify for the study. These studies may include the following: 1) x-rays of the brain, chest and abdomen; 2) blood tests; 3) testing your blood for the antibodies to human immunodeficiency virus (HIV) that causes AIDS. If I am found to have these antibodies, I may not participate in this study. If I do qualify, I will undergo a series of procedures as part of the treatment.

I understand that the first step will require the surgical removal of one of my metastatic tumors in order to grow the tumor cells in a test tube. This surgical procedure will probably be done under local anesthesia but may require general anesthesia. My tumor cells will then be modified by insertion of genetic material using a mouse virus which will result in their production of IL-4, a protein normally made by the body's immune system which can help stimulate immune cells.

After making these modified tumor cells, they will be irradiated in the test tube and then injected into the skin of my thigh or underarm area in order to stimulate an immune reaction in nearby lymph nodes against my tumor. The injections will be done with a standard needle and syringe. Approximately 7 to 10 days later, the lymph nodes adjacent to the injected skin sites will be surgically removed. This procedure will probably be done under local anesthesia. The lymph nodes will be taken to the laboratory where the cells from them will be further stimulated in the test tube and grown into larger amounts. After 2 to 3 weeks, the lymph node cells should have grown sufficiently enough to transfuse them back into me along with the administration of IL-2. This part of the treatment will require hospitalization for several days. I am aware that it will be necessary to place an intravenous catheter through a vein either on the upper chest or in the neck that will be threaded into a central vein in my body. This intravenous catheter is placed under local anesthesia at the bedside. The lymph node cells will be infused once through this intravenous catheter over a 30 minute period and will be followed by 15 minute IL-2 infusions given every 8 hours for up to 15 doses. The IL-2 is a protein normally produced by the body which is given to allow the injected lymph node cells to remain alive.

I understand that at different times in the study, biopsies of superficial tumors or the skin injection sites may be performed. These biopsies would be performed under local anesthesia and would not occur more than two times.

RISKS AND SIDE EFFECTS

I know that there are potential side effects and risks to these procedures which are summarized below.

Surgical removal of tumor and lymph nodes:

These procedures may be associated with discomfort which should be minimized by an appropriate anesthetic agent. It is possible I may develop bleeding or an infection at the site of the procedure, I will receive a separate surgical consent document for these procedures.

Skin injection with gene-modified tumor cells:

I will be injected with gene-modified tumor cells into the skin in an attempt to stimulate an immune response against my tumor. This may cause mild discomfort at the site of injection. One possible side effect may be growth of tumor at the skin injection site. The tumor cells will have been irradiated prior to the injection procedure in an effort to avoid this problem. If tumor does grow at the injection sites, they will be removed surgically in order to minimize problems in these areas.

I understand the potential risks associated with receiving cells modified by this gene. The genetic material was inserted into my tumor cells with the use of a mouse virus. I have been informed that even though the mouse virus used to insert the gene into my tumor cells cannot grow and is considered harmless to me, it is possible that events could occur within the cell that allow the virus to grow. To minimize this possibility, the virus used to modify the cells will be tested extensively prior to use. I am

aware that there is a remote chance that the use of this virus may cause a new cancer. Because this procedure is relatively new, it is possible that despite extensive precautions, other unforeseen problems may occur including the very remote possibility of death.

The gene-modified tumor cells I will receive are designed to produce an immune hormone called IL-4. This hormone is naturally produced in the body in small amounts. However, the gene-modified tumor cells which are injected into my skin may produce too much IL-4. This might lead to side effects which include fatigue, flu-like symptoms, diarrhea, nausea, loss of appetite, headache and fluid retention. I will be monitored for this possible side effect by obtaining blood samples on a periodic basis to measure IL-4 levels. If it becomes apparent that I am experiencing problems related to IL-4 production by the tumor cells, they will be surgically removed.

Lymph node cells/IL-2 infusions:

For the administration of my lymphocytes it will be necessary to place an intravenous catheter through a vein either on the upper chest wall or in the neck that will be threaded into a central vein in the body. The risks involved in having such a central venous catheter include collapse of the lung, infection, or development of a blood clot in the vein. Infusion of the cells into my vein is performed over a 30 minute period. The administration of these cells may cause side effects including fever, chills and some shortness of breath but I have been informed that these effects last for only a few hours.

I am aware that the administration of IL-2 can cause significant side effects. These include weight gain from fluid retention, shortness of breath, a drop in blood pressure, infections, nausea, diarrhea, confusion, abnormal heart beats, and abnormalities of liver and kidney function. I have been informed that these side effects require close monitoring in the hospital. If any serious side effect occurs I understand the treatment will stop and appropriate medical actions will be taken. I am aware that treatment with IL-2 may cause my death. Less than 1% of patients who have received IL-2 have died as a result of the treatment.

FOLLOW-UP

After I receive the treatment, I will be required to return to the University of Michigan Medical Center for follow-up studies approximately four and eight weeks after hospital discharge. Tests used to decide if my tumor responded to the therapy will be similar to those I had before beginning the therapy. I will also undergo skin testing at these time intervals as well as prior to treatment. Some of these skin tests will be done to determine if I react to my own tumor cells. This will involve the injection of irradiated tumor cells in my skin. If my disease worsens after treatment in this protocol, I will be eligible for other protocols and will receive treatment as indicated by my disease or referred elsewhere for such treatment. Because this form of therapy is new, unanticipated side effects that may cause my condition to deteriorate could be encountered. Therefore, I agree to be monitored for such side effects for the rest of my lifetime by the research investigators of this study..

By two months after treatment, if I have evidence of tumor shrinkage or my tumors remain unchanged it is possible that additional treatment will be offered. I understand repeat treatment would consist of receiving additional lymph node cells which had been frozen and stored from my initial procedures; along with the administration of IL-2. Repeat treatments would not entail any further surgical procedures to remove tumor or lymph nodes. If lymph node cells are not available, I will be offered treatment with IL-2 alone. My physicians feel that the risks of my disease are much greater than the risks of the treatment as outlined above. Furthermore, my physicians have considered my individual situation and have concluded that, at this time, no other therapeutic approaches such as surgery, radiation therapy or other chemotherapeutic treatments are clinically indicated as being more effective. At some later time, should these alternatives be clinically indicated, they will be discussed with me because this study does not preclude their use.

Due to the nature of this study which involves the genetic alteration of some of my tumor cells, I have been informed that vital information could be obtained from an autopsy examination in the event of death at any future time. I grant permission for this examination. However, I reserve the option to

withdraw my consent at any time during treatment or follow-up with no loss of benefits or disruption in my care.

POTENTIAL BENEFITS FOR PARTICIPANTS

I understand that there may be no direct benefit to me from study participation, and there is a possibility my condition may become worse. In the past, lymphocyte and IL-2 infusions have resulted in some tumor shrinkage in some individuals but not in others. Even those tumors that exhibit shrinkage may show regrowth after a short period of time. A better understanding of immunity to cancer may be gained by my participation that would benefit other patients.

ALTERNATIVE THERAPIES

There are no known cures for patients with my disease. I am aware that alternative treatments available to me include chemotherapy or other experimental treatments. The option also exists to receive no treatment at this time.

OTHER PERTINENT INFORMATION

1. Confidentiality. I understand that when results of a study such as this are reported to medical journals or at meetings, the identification of those taking part is withheld. Medical records are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act. A qualified representative of the National Institutes of Health may inspect patient and study records. I understand that this experimental treatment may attract attention from the media. I have been informed that every effort to protect my confidentiality will be made. Because of media interest, however, I realize there is a significant chance that information concerning me and my treatment may appear publicly without my consent.

2. Policy regarding research-related injuries. I understand that in the unlikely event a physical injury resulting from research procedures, the University will provide first-aid medical treatment. Treatment of injuries or side effects directly related to this experimental treatment will be provided at no cost to me. Additional medical treatment will be provided in accordance with the determination by the University of responsibility to provide such treatment. However, the University does not provide compensation to a person who is injured while participating as a subject in research.

3. I will not be paid to take part in this study.

4. Laboratory tests and scans will be ordered to determine my eligibility for study and whether I have had a response to the treatment. Items which are not covered by insurance which relate to this research protocol will be free. The cost of tests and treatments unrelated to this study will be charged for. I understand that insurance coverage cannot be guaranteed for all tests and treatments.

5. This consent form does not include consent relating to the risks of any surgical procedures. Any surgical procedures performed will require a separate consent form.

6. This research may have unforeseeable risks to an embryo or fetus. If I could potentially father a child or bear a child; I will not participate in this research study unless I am employing a medically acceptable form of birth control (contraception).

7. I am free to withdraw my consent to participate in this study at any time during treatment or follow-up and seek care from any physician with no loss of benefits or disruption in my care.

If you have questions pertinent to this research, you should contact Alfred E. Chang, M.D., at 313/936-4392.

If you feel that you have a research-related injury, contact Alfred E. Chang, M.D., at 313/936-4392.

The following numbers are for your use if medical problems develop during treatment:

Office:	313/936-4392
Clinical Research Center:	313-936-8090
Voice Mailbox:	313/936-4392 (after office hours)
Doctor or Nurse:	313/936-6267 (This is hospital paging operator. Ask for the physician on call for Surgical Oncology).

Questions on my rights as a patient may be directed to Ann Munro in the Patient/Staff Relations Office at 313/763-5456

I have fully explained to the patient, _____ the nature of the treatment program described above and such risks involved in its performance.

_____
Physician's Signature

I have been fully informed as to the procedures to be followed including those which are investigational, and have been given a description of the attendant discomforts, risk, and benefits to be expected, and the appropriate alternative procedures. I realize that since my participation is voluntary, I can refuse this treatment without in any way prejudicing my future medical care. In signing this consent form, I agree to this method of treatment, and I understand that I will receive the best supportive care even if not receiving this protocol treatment. I also understand that my doctors can stop my treatment on this protocol if they feel the risks in my case have increased, or to exceed the potential benefits to me. I understand, also, that if I have any questions at any time, they will be answered. I have retained a copy of this consent form.

I understand that the University will provide first-aid medical treatment in the unlikely event of physical injury resulting from procedures. Treatment of injuries or side effects directly related to experimental treatment will be provided in accordance with the University's determination of its responsibility to do so. The University does not however, provide compensation to a person who is injured while participating as a subject in research.

_____
Signature of Patient

_____
Date

_____
Witness

11/93

BIOGRAPHICAL SKETCH

CVs

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Alfred E. Chang, M.D.	Professor of Surgery

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Dartmouth College, Hanover, NH	BA	1971	Chemistry
Harvard Medical School, Boston, MA	MD	1974	Medicine
Duke University Medical Center, Durham, NC		1974-1979	Surg. Int/Res
National Cancer Institute, Bethesda, MD		1976-1979	Surgical Oncology
Hosp. Univ. of Penn., Philadelphia, PA		1979-1982	Surgery Resident

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Professional Experience:

1982-1987 **Senior Investigator**, Surgery Branch, NCI, Bethesda, MD
1984-1987 **Asst.Prof.Surg.** Uniformed Services of the Health Sciences, Bethesda,MD
1988-Pres. **Director**, GI Tumor Program, Univ. of Mich. Cancer Center
1988-Pres. **Associate Director**, Clinical Affairs, Univ. of Mich. Cancer Center
1988-1992 **Assoc. Prof., Surg.**, University of Michigan, Ann Arbor, MI
1992-Pres. **Professor of Surg.,** University of Michigan, Ann Arbor, MI
1988-Pres. **Chief, Div. of Surg. Oncology,** Univ. of Mich., Ann Arbor, MI

Academic Activities:

Member, Editorial Board, Surgical Oncology, Member, Editorial Board, Annals of Surgical Oncology, Reviewer, American Cancer Society, Clinical Study Section, grants review program.

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patients with advanced cancer using lymphokine activated killer cells and interleukin-2 or high dose interleukin-2 alone. N Engl J Med 316:889-897, 1987.

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HUMAN GENE TRANSFER PROTOCOL

9312-066 Sorscher, Eric J. and Logan, James L.; University of Alabama, Birmingham, Alabama; *Gene Therapy for Cystic Fibrosis Using Cationic Liposome Mediated Gene Transfer: A Phase I Trial of Safety and Efficacy in the Nasal Airway.*

Date of RAC Approval: 12/3/1993

SCIENTIFIC ABSTRACT

Cystic fibrosis (CF) is a common, lethal, inherited disease among Caucasian children and young adults. While the pathophysiology of CF includes many organ systems (e.g., gastrointestinal, reproductive, endocrine) the predominant cause of death is respiratory failure. Hyperviscous respiratory secretions and related chronic pulmonary infections lead to scarring and fibrosis of the lungs, deteriorating pulmonary function, and death, with an average CF life expectancy of approximately 29 years. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The CFTR functions, at least in part, as an epithelial chloride channel which resides in the apical membranes of exocrine gland epithelial cells. It has been suggested that the fluid and electrolyte content of normal respiratory mucous is dependent upon CFTR chloride channel activity, and that failure to secrete chloride by CF cells results in diminished hydration of mucous, sputum hyperviscosity and the pulmonary sequelae of the disease.

Transfer of normal human CFTR to cells possessing the CF ion transport phenotype (i.e., with defects in Cl^- transport), or into the respiratory epithelium of CF mice leads to re-establishment of CFTR mediated chloride transport. Findings of gene transfer mediated correction of the CF bioelectric defect in these model systems, together with the failure of current therapies to allow CF patients to live beyond young adulthood, indicates the need for new approaches to CF therapy and the possibility of gene transfer-based protocols in the disease. Five gene transfer based protocols using recombinant adenovirus to deliver functional CFTR to limited regions of CF respiratory epithelium *in vivo* have been approved previously for use in humans by the Recombinant DNA Advisory Committee of the N.I.H. Evaluation of the safety and efficacy of adenovirus-based CFTR delivery is currently in progress.

The present protocol is intended to evaluate an alternative, cationic liposome-based mechanism of delivery of normal CFTR to nasal respiratory epithelia of CF patients. The advantages of lipid-based gene transfer are evident, and may include 1) minimal toxicity, 2) less antigenicity than viral DNA delivery vectors, and 3) efficient gene transfer even to non-replicating cells. The nasal airway epithelium is an ideal model for gene transfer of this type, since this epithelium exhibits a CF bioelectric defect and is easily accessable for studies of both safety and efficacy of DNA/vector administration. In addition, the use of this site in CF patients greatly minimizes the risk of serious complications related to gene transfer to the lower airways.

NON-TECHNICAL ABSTRACT

Cystic fibrosis (CF) is a common inherited disease, effecting about 1 in 1600 Caucasians. Most patients with cystic fibrosis die before age 30. The disease can effect many parts of the body (including the pancreas, reproductive glands, and sweat glands), but the cause of death is usually due to lung failure from thick secretions and chronic infection of the lungs.

Human genes carry functions which are necessary for healthy human life. Cystic fibrosis is caused when one of these genes, called the cystic fibrosis gene, cannot function properly. As a result of this dysfunction, tissues in the bodies of CF patients (and the cells which are the building blocks of these tissues) cannot move salts properly. For example, the high content of salt in the sweat of CF patients, and the thick respiratory mucous in the lungs of CF patients, have both been associated with difficulties moving salts such as sodium and chloride.

The purpose of this study is to determine whether normal, functional copies of the CF gene can be delivered to the cells lining the nose of CF patients. This study will use small amounts of molecules of fat (also called "lipids") to carry the normal gene into these cells.

This study has two goals. The first goal is to determine whether lipids can be used to safely carry the normal gene into the cells lining the nose. The second goal is to determine whether after receiving the normal CF gene, the inside of the nose will develop an improved ability to move salts.

The use of genes to treat human diseases has been called "gene therapy". Because the serious complications of CF occur in parts of the body other than the nose (for example, in the lungs and pancreas), it is unlikely that the delivery of a normal CF gene to the inside of the nose will make participants in this study healthier. On the other hand, the overall goal of this research study will be to allow the possibility of attempting the same type of normal gene delivery to CF lungs, with the hope of developing better treatments for cystic fibrosis.

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GENE THERAPY FOR CYSTIC FIBROSIS USING CATIONIC LIPOSOME MEDIATED GENE TRANSFER: A Phase I Trial of Safety and Efficacy in the Nasal Airway

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PROTOCOL

I.A. PURPOSE OF THE PROJECT

Cystic fibrosis (CF) is a common, lethal, inherited disease among Caucasian children and young adults. While the pathophysiology of CF includes many organ systems (e.g., gastrointestinal, reproductive, endocrine) the predominant cause of death is respiratory failure (1). Hyperviscous respiratory secretions and related chronic pulmonary infections lead to scarring and fibrosis of the lungs, deteriorating pulmonary function, and death, with an average CF life expectancy of approximately 29 years. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (2-4). The CFTR functions, at least in part, as an epithelial chloride channel which resides in the apical membranes of exocrine gland epithelial cells (5-10). It has been suggested that the fluid and electrolyte content of normal respiratory mucous is dependent upon CFTR chloride channel activity, and that failure to secrete chloride by CF cells results in diminished hydration of mucous, sputum hyperviscosity and the pulmonary sequelae of the disease.

Transfer of normal human CFTR to cells possessing the CF ion transport phenotype (i.e., with defects in Cl^- transport), or into the respiratory epithelium of CF mice leads to re-establishment of CFTR mediated chloride transport (9-11). Findings of gene transfer mediated correction of the CF bioelectric defect in these model systems, together with the failure of current therapies to allow CF patients to live beyond young adulthood, indicates the need for new approaches to CF therapy and the possibility of gene transfer-based protocols in the disease. Five gene transfer based protocols using recombinant adenovirus to deliver functional CFTR to limited regions of CF respiratory epithelium have been approved previously for use in humans by the Recombinant DNA Advisory Committee of the N.I.H. (12). Evaluation of the safety and efficacy of adenovirus-based CFTR delivery is currently in progress.

The present protocol is intended to evaluate an alternative, cationic liposome-based mechanism of delivery of normal CFTR to nasal respiratory epithelia of CF patients. The advantages of lipid-based gene transfer are evident, and may include 1) minimal toxicity, 2) less antigenicity than viral DNA delivery vectors, and 3) efficient gene transfer even to non-replicating cells (11, 13-15). The nasal airway epithelium is an ideal model for gene transfer of this type, since this epithelium exhibits a CF bioelectric defect and is easily accessable for studies of both safety and efficacy of DNA/vector administration (12, 16-18). In addition, the use of this site in CF patients greatly minimizes the risk of serious complications related to gene transfer to the lower airways.

I.B. BACKGROUND

I.B.1. Clinical Aspects and Standard Care.

Cystic Fibrosis is an autosomal recessive disease with a prevalence of approximately 1:1600 live births among Caucasian individuals of European descent (1). While the modern hallmark of the disease is respiratory failure, the disease often presents as growth retardation in infants or young children. Poor growth in most cases is attributable to exocrine pancreatic insufficiency and chronic dietary malabsorption. Histopathologic examination of CF pancreatic specimens demonstrates extensive destruction including dense fibrosis with formation of large cystic structures (hence the full name of the disease, "cystic fibrosis of the pancreas"). The pathologic changes in the pancreas have been ascribed to primary abnormalities in content and/or

hydration of exocrine pancreatic secretions, leading to pancreatic failure and inadequate levels of the pancreatic digestive enzymes crucial for absorption of a normal diet. Clinically, CF pancreatic insufficiency can be managed by administration of exogenous pancreatic dietary enzymes (for example, enzymes derived from porcine [Ultrase or Pancrease] sources) which are taken with meals. Prior to the development of exogenous pancreatic enzyme supplementation and other nutritional interventions, most CF patients died in infancy or early childhood of malnutrition.

The ability to treat the nutritional aspects of CF led to an improved life expectancy of CF patients, and also to the need for improved therapies for the pulmonary sequelae of the disease. The lungs of CF patients undergo progressive fibrosis and scarring which may begin very early in life. Respiratory mucous in CF is thick, viscous and very difficult for patients to expectorate. Difficulty in clearing the respiratory mucous is associated with chronic pneumonia, including some forms of pneumonia caused by antibiotic-resistant organisms which are nearly pathognomonic for individuals with CF lung disease (e.g., respiratory colonization and pneumonia due to mucoid strains of multiply-resistant Pseudomonas aeruginosa). Tenacious secretions and chronic respiratory infection lead to diminished pulmonary function (e.g., decreased FVC, decreased FEV_1), loss of lung volume, and progressive mixed obstructive and restrictive lung disease. Most patients suffer from chronic progressive hypoxemia and air-hunger, and ultimately require repeated hospitalizations for intravenous broad-spectrum antibiotics to treat chronic pneumonia, as well as bronchodilators, oxygen supplementation, and chest physical therapy designed to mobilize inspissated pulmonary mucous and to improve aeration. Despite these standard measures of care, most CF patients die in young adulthood of respiratory failure. The current average life expectancy for a CF patient is 29 years (male) or 28 years (female). Other organ systems are commonly effected by cystic fibrosis. For example, newborns with CF may develop meconium ileus, an obstruction of the bowel due to thick and inspissated neonatal stool. Abnormalities in secretions within exocrine glands of the female and male reproductive tract have been implicated as contributing to the high incidence of infertility among CF patients of reproductive age (greater than 95% of adult CF males are infertile and have significant azoospermia; a large number of adult CF females are also infertile). Exocrine pancreatic structural damage may result in loss of endocrine pancreatic islets and clinical diabetes, with the requirement for insulin administration. Chronic lung disease may secondarily lead to life-threatening hemoptysis requiring surgical intervention, or to right heart failure, which may be managed by appropriate diuretic or vasodilator therapy.

I.B.2 Bioelectric abnormalities and relationship to CFTR.

Prior to identification of the CF gene, the finding that sweat levels of sodium and chloride were elevated in CF led to the suggestion that the disease was caused by a primary defect in ion transport (5,19). Studies of isolated, perfused human sweat ducts demonstrated defects in chloride transport in CF. Similarly, respiratory epithelial cells taken from CF patients lacked a cyclic AMP (cAMP) stimulated component of chloride transport. Since the discovery of the CFTR in 1989 (2-4), compelling evidence has accumulated that the CFTR functions, at least in part, as an apical membrane, cAMP stimuable chloride channel. Expression of CFTR in cells taken from CF patients, as well as in numerous heterogenous cell types, leads to the appearance of a cAMP dependent chloride channel (9,10,20-22). Antisense experiments designed to eliminate CFTR lead to diminished Cl^- transport in sweat duct cells, and point mutations or

domain deletions in recombinant CFTR lead to alterations in single chloride channel currents compatible with the notion that CFTR directly functions as a chloride channel (23,24). Mice obtained after inactivation of the murine CFTR exhibit defects in cAMP-stimulated chloride transport and correction of these abnormalities has been reported following transfer of the normal human CFTR in vivo (11,25). In addition to its role as a chloride channel, other CFTR functions have been defined, including a role in regulating epithelial amiloride-sensitive sodium transport (16-18), calcium dependent chloride transport (26), and trafficking of epithelial endocytic and exocytic vesicles (27).

I.B.3. Pathophysiology of CF.

Over 300 mutations in CFTR have been described which are associated with clinical CF, and evidence suggests heterogenieity in the molecular pathogenesis of the disease. For example, the most common CFTR mutation, deletion of a phenylalanine residue at CFTR position 508 (i.e., Delta F508), appears to cause biosynthetic arrest of the full-length CFTR, with premature degradation of CFTR in the endoplasmic reticulum and failure to properly insert the protein in the apical membrane (28,29). The second most common CFTR mutation (a glycine → aspartic acid replacement at CFTR position 551, i.e., G551D) is associated with normal cellular processing, but may cause disease by interfering with nucleotide binding and activation of CFTR (30). Other mutations cause premature stop codons within the protein (31,32).

The fluid and electrolyte content of normal respiratory mucous is dependent upon CFTR chloride channel activity, and it has been suggested that failure to secrete chloride by CF epithelial cells results in diminished hydration of mucous, sputum hyperviscosity, and the pulmonary sequelae of the disease. This model for disease pathogenesis, together with an improved understanding of the molecular physiology of CF, has lead to several novel therapeutic approaches. Drugs designed to activate chloride secretion into the airways despite defective CFTR (e.g., extracellular nucleotides [18]), and methods to allow normal biosynthetic processing or activation of the Delta F508 CFTR (e.g., methylxanthines, temperature modulation)(21,33,34) are currently undergoing in vitro or in vivo evaluation. Associated functions of CFTR, such as the regulation of amiloride sensitive sodium transport, may also contribute to clinical disease. For example, potential difference measurements across the airway epithelium (see below) indicate excessive airway sodium reabsorption through an amiloride inhibitable pathway. Because excess sodium uptake from the airways could result in excessive removal of airway water and dessication of respiratory mucous, mechanisms for blocking airway sodium uptake in CF are currently under investigation. The use of aerosolized amiloride to treat CF pulmonary disease is currently undergoing Phase II/III multi-center human trials.

I.B.4. Gene Transfer-Based Therapy of CF Lung Disease

The findings of gene transfer mediated correction of the CF defect in cultured cells and in CF mice, together with the failure of all current therapies to allow CF patients to live beyond young adulthood, indicates the need for new approaches to CF therapy and the possibility of gene transfer-based protocols in the disease. Five protocols using recombinant E1 deleted adenovirus to deliver wild type CFTR to the nasal and lower airway epithelium of CF patients have already been approved by the Recombinant DNA Advisory Committee of the NIH (12). Recombinant adenovirus has been shown to efficiently deliver reporter genes to respiratory epithelium of cotton rats, to human airway epithelium grown in vitro or in a reconstituted

tracheal grafts, and to certain non-human primates (35-38). Considerable pulmonary inflammation has been associated with administration of high-titer E1 deleted adenovirus to non-human primates. Endpoints for evaluation of efficacy in these studies of adenoviral-based CFTR delivery include 1) bioelectric measurements of ion transport across intact epithelium, 2) ion transport measurements in respiratory cells cultured from CF patients following gene transfer (either using isotope efflux, halide sensitive intracellular reporter dyes, or growth in an epithelial layers with mounting in Ussing Chambers) and 3) studies of wild-type CFTR mRNA and protein expression. Further evaluation is in progress concerning safety and efficacy of E1 deleted adenovirus as a gene delivery vehicle in the therapy of CF.

Because adenoviral genes exist episomally in host cells, gene transfer using these vectors leads to transient expression. It is anticipated that periodic pulmonary re-administration of adenovirus harbouring CFTR would be necessary in order to maintain functional activity. Two other viral vectors offer the possibility of chromosomal integration and possibly much more prolonged expression of CFTR within respiratory epithelium. Retroviral based gene transfer has already been used to correct the bioelectric defect in CF cells in vitro (9). However, the relatively low titers currently obtainable for recombinant retroviral vectors (approximately 10^6 infectious viral particles/ml of culture medium) limits the use of retroviral vectors for direct administration to human pulmonary epithelium, where the number of pulmonary epithelial cells exceeds obtainable retroviral titers by serveral orders of magnitude (39,40). Furthermore, because of a very low proliferative rate of pulmonary airway epithelium, and since retroviral chromosomal integration requires active cell division, retroviral based CFTR delivery is further limited in this context.

In addition to retrovirus, adeno-associated virus (AAV) has the potential to allow gene integration within host chromosomes and has been used to deliver CFTR in vitro and in vivo (41,42). Relatively low titers of recombinant AAV (10^6 pfu/ml) may limit the usefulness of this vector in CFTR gene transfer to human lungs in vivo, although newer methods of generating AAV are rapidly evolving. There is also evidence suggesting that AAV integration is independent of cell replication and may occur at a site on chromosome 19, possibly decreasing the theoretical risk of undesirable toxicity which could be associated with random chromosomal integration.

I.B.5. Cationic Liposome-Based Delivery of CFTR

Increasing evidence indicates that cationic liposomes are capable of transferring foreign genes to pulmonary epithelium in vitro and in vivo. Stribling, et al., (13) used a 1:1 molar ratio of cationic:neutral lipid to generate DNA/lipid complexes suitable for in vivo gene delivery to the mouse airway after aerosolization. Liposomes generated as small unilamellar vesicles suitable for in vivo gene transfer (100 μm diameter) containing the cationic lipid DOTMA (N-[1-(2,3-dioleoyloxypropyl)]-3-trimethylammononium-propane) and the neutral lipid DOPE (dioleoyl phosphatidylethanolamine) were complexed with a chloramphenicol acetyltransferase reporter plasmid. Twelve mg of the plasmid DNA were administered as a nebulized aerosol per mouse per day. No histologic evidence of toxicity was observed, and high levels of reporter gene activity were obtained in the animals lungs both by quantitative enzymatic assay and by immunocytochemical localization of the reporter gene product. The majority of airway epithelial cells and alveolar lining cells were transfected in vivo; nearly all conducting airways showed intense and uniform staining, often approaching 100% gene transfer efficiency. Yoshimura, et

al., (14) have shown that direct instillation of DOTMA/DOPE/DNA conjugates into the mouse trachea can mediate pulmonary expression of both the E. coli Lac Z gene and firefly luciferase. After conjugation to lipid, up to 1.4 mg of plasmid DNA in 200 μl total volume could be safely administered per mouse. Airway epithelium was again shown to be the major target of gene transfer, and reporter gene expression lasting at least four weeks was observed by this approach. In addition, lipid mediated gene transfer of CFTR was established using PCR amplification of reverse transcribed pulmonary mRNA from experimental animals. Hyde, et al. (11) have shown that DOTMA/DOPE/DNA conjugates can mediate functional transfer of CFTR to epithelial cells in vivo with reversal of the ion transport defect in transgenic CF mice. These results have significance, since they represent the first in vivo correction of the CF ion conductance abnormality, and illustrate the feasibility of cationic liposome mediated gene transfer for cystic fibrosis therapy. Finally, Zhu, et al., (15) recently reported high levels of gene transfer in the lungs, lymph nodes, spleen and bone marrow of adult mice for up to nine weeks following a single IV injection of a DOTMA/DOPE/DNA conjugate without apparent toxic effects.

The mechanism by which cationic liposomes mediate gene transfer and expression is not well understood, and in vitro efficiency of the technique is unpredictable. As a result, studies of the generalizability of this phenomenon both in vitro and in vivo are appropriate prior to designing human-based trials. We have evaluated the efficiency of three different cationic lipids (in addition to DOTMA, both DOTAP (1,2-Dioleoyl-3-trimethylammonium-Propane) and DMRIE (1,2-dimyristyloxypropyl-3-dimethylhydroxyethylammonium Bromide) in a 1:1 molar ratio with DOPE were used) to confer expression of reporter genes (E. coli Lac Z and firefly luciferase) and functional CFTR (see II.A. Preclinical Data). These experiments represent the first report of in vivo cationic lipid-based gene transfer to animals other than mice using a lipid formulation other than DOTMA/DOPE. Our results indicate that cationic lipid mediated gene transfer is generally efficacious within mammalian pulmonary epithelium. These results further support the establishment of human-based trials of cationic liposome mediated transfer of CFTR to limited areas of respiratory epithelium in vivo.

Efforts aimed to develop human-based trials of lipid/DNA conjugates to correct the CF defect in airways of patients with CF now seem appropriate. Because neither the safety nor efficacy of gene transfer of CFTR into the human lung by any vector system has yet been established, the importance of aggressively investigating promising alternative means of CFTR delivery to pulmonary epithelium is evident. The use of lipid/DNA conjugates and CFTR gene transfer could have substantial advantages over use of the recombinant adenoviral or other recombinant viral strategies which have been proposed for use in CF gene transfer trials. These advantages may include 1) minimal toxicity, 2) less antigenicity than viral DNA delivery vectors, and 3) efficient gene transfer even to non-replicating cells. Furthermore, unlike viral based vectors, lipid based delivery carries no risk of infection, replication or transmission of virus and no anticipated risks of malignant transformation (a theoretical concern with the use of retroviral and adenoviral vectors) (43-46).

I.B.6. Respiratory Epithelial Cell Targets for CF Gene Transfer; Use of Nasal Respiratory Epithelium as a Model for the CF Bioelectric Defect

The approach in current protocols for gene transfer in the therapy of CF lung disease is to directly administer vectors containing CFTR to pulmonary epithelium in vivo (12). Previous studies using in situ hybridization and immuno-histochemistry (with antibodies raised against

human CFTR) indicate that native CFTR expression occurs within airway epithelial cells as well as the submucosal glands of the lung (47). Extensive biophysical, histopathologic, as well as cellular localization studies indicate that the pulmonary epithelial cells, and particularly cells lining the airways, are the appropriate target for initial CFTR gene transfer experiments. In addition, based upon experiments in which CF cells lacking the normal CFTR have been mixed with CFTR-corrected cells, it has been estimated that between 3-6% of cells in an epithelial layer must be corrected in order to begin to obtain normalization of the CF transport defect (48). This data provides an estimate of a lower limit of the proportion of cells within a monolayer which must be corrected in order to achieve at least some normalization of CFTR dependent ion transport.

In analogy to previous gene transfer protocols for CF, the intent of this project will be the direct administration of CFTR to airway epithelium (in this case using lipid/DNA conjugates) in vivo. The predominant target cells will be the surface epithelial cells within the nasal airway. In CF, the nasal epithelial layer exhibits the same ion transport defects that are observed in the lower airways, and thus forms an ideal model system for study of potential therapies for CF lung disease in vivo. Potential therapies for cystic fibrosis using pharmacologic agents (amiloride, adenosine) as well as the approved gene transfer based strategies, have used nasal epithelium as a testing ground in the past (12,16-18). The nasal epithelium is easily accessible to 1) administration of therapeutic agents, 2) measurement of the CF bioelectric defect, and 3) biopsy/scrapings/washings, etc., to evaluate inflammation or other toxicity. In addition, use of the nasal airway allows potential therapies for CF lung disease to be tested without placing CF patients at the risks associated with administration of experimental agents to the severely compromised pulmonary airways already present within this patient population.

The optimal level of expression of CFTR within individual respiratory target cells is not known. It is likely that endogenous levels of CFTR mRNA are quite low (on the order of 1-2 mRNA molecules per cell [49]), and it is anticipated that viral or plasmid-based gene transfer may result in much higher expression levels than those found endogenously. No CFTR related toxicity has been observed in CF or other cells in culture which over-express the CFTR protein. In addition, studies in transgenic mice in which human CFTR is markedly over-expressed in pulmonary epithelial (and other) cells have not indicated any toxicity attributable to production of the human CFTR (50). There is a theoretical possibility that expression of the wild-type human CFTR in CF patients could lead to development of anti-CFTR antibodies and other inflammatory responses directed against cells expressing the wild-type protein. For example, if the common Delta F508 CFTR is not normally present on the cell surface in CF patients, it is possible that wild-type CFTR in a homozygous Delta F508 patient could produce an immune response specific for human CFTR. However, this has not been reported either in transgenic mice expressing human CFTR, or in CF mice or rats expressing CFTR following lipid mediated gene transfer (11,50, also see below).

I.B.7. Cationic Lipid Formulations for Use in Gene Transfer

Several cationic lipid formulations can be used as gene transfer vectors to deliver plasmid DNA in vitro, although certain of these appear to show considerably enhanced efficacy in reporter gene assays in certain cell types. Felgner, et al., (51) described the use of a 1:1 molar mixture of DOTMA with DOPE (lipofectin) to deliver a plasmid DNA containing a chloramphenicol acetyl transferase reporter gene construct to COS-7 simian kidney cells. This

formulation has subsequently been shown to be a highly versatile gene delivery vector, with capacity to mediate exogenous gene expression in over 100 cell lines in vitro, as well as to deliver mRNA, antisense oligonucleotides, and protein transcription factors, for example, in active form to eukaryotic cells (52). In addition, as described above (Section I.B.5.) DOTMA/DOPE has been used to directly deliver both reporter genes (luciferase, Lac Z, and chloramphenicol acetyl transferase) and CFTR directly to airway epithelial cells following either direct installation into the mouse trachea, aerosolization, or, in one study, intravenous administration. New generation cationic lipid formulations have been found to be substantially superior to DOTMA/DOPE. Felgner evaluated a large series of cationic lipid formulations for their ability to mediate gene transfer to COS-7 cells in vitro under a variety of lipid:DNA ratios, complexed to two different neutral lipids (DOPE and DOPC, dioleoylphosphatidyl choline), and in the presence and absence of chloroquine (53, included in appendix). One of these cationic lipid formulations [a 1:1 molar mixture of DMRIE (1,2-dimyristyloxypropyl-3-dimethyl-hydroxy ethyl ammonium bromide) with DOPE] led to a several-fold enhancement of ß-galactosidase expression when compared with lipofectin in vitro, with no enhanced cytotoxicity.

The mechanisms by which cationic liposomes mediate gene transfer, and the means by which cells process and express exogenously delivered plasmid DNA are not well understood. Positively charged lipid head groups have been necessary in all effective liposome formulations and a polyamine moiety in the head group has recently been reported to enhance DNA expression by 3 to 30-fold in certain cell types (compared with lipids containing a head group with a single positive charge). In addition, combining cationic and neutral lipids (such as DOPE) within liposomes appears to consistently enhance reporter gene expression using cationic lipids. It has been proposed that neutral and cationic lipids form a stable liposome in solution with positive charges in many of the externally-directed polar head groups. In this model, the positive charges on the surface of the liposomes serve two purposes:

1) To form ionic bonds with the negatively charged phosphates within plasmid DNA backbone (i.e., to "stud" the plasmid DNA molecule with liposomes), and
2) To bind negatively charged cell surface molecules, thereby bringing the lipid/DNA conjugates in close proximity to the cell membrane.

In this scheme, the highly fusogenic neutral lipids forming part of the outer bilayer of the liposome would mediate fusion of the liposome with the cell membrane, and in the process allow DNA to enter the cytoplasm (53,54).

Two protocols (55) have previously been approved by the Recombinant Advisory Committee of the NIH for the use of lipid mediated gene transfer in the treatment of human disease. Both protocols employ injection of lipid/DNA complexes directly into palpable melanoma tumor nodules in order to express a foreign HLA antigen (HLA B7) in a small fraction of tumor cells. The intent in these studies is to enhance immune mediated clearance of the tumors. One of the lipid formulations approved in this capacity is DMRIE/DOPE. As described below, DMRIE/DOPE has also shown efficacy as a gene delivery vehicle for transferring CFTR to human and other mammalian airway epithelia, and will be proposed in this trial for use in CFTR delivery to a small area of human nasal epithelium.

II.A. Preclinical Data

II.A.1. Cationic Lipid Mediated Gene Transfer to Human Respiratory Epithelium in vitro.

Gene transfer was demonstrated in vitro using both E. coli Lac Z and firefly luciferase genes. As shown in Figures 1 and 2, DOTMA/DOPE and DMRIE/DOPE were each able to mediate high level reporter gene expression in both CF ($2CFSME_{0-}$) and non-CF ($16HBE\text{-}14_{0-}$) human respiratory epithelial cells. DNA or lipid alone in control experiments led to negligible luciferase activity (less than 200 relative light units per measurement). In addition, 5% of cells transfected with Lac Z plasmid DNA showed evidence of gene expression. No Lac Z reporter gene expression was observed in control cells (untreated) or cells treated with lipid alone or DNA alone (results not shown).

II.A.2. Efficiency of DNA Delivery in vitro using Cationic Liposomes.

Figure 3 shows the results of experiments in which fluorescently labeled plasmid DNA was delivered using cationic liposomes, in order to determine the efficiency of gene delivery in vitro. In three cell lines, including the $2CFSME_{0}^{-}$ line, nearly 100% of cells exhibited nuclear uptake of plasmid DNA within four hours of transfection. In an additional series of experiments fluorescent liposomes {using 1-oleoyl-2 [6 (7-nitro-2-1,3-benzoxadiazol 4 yl) amino] caproyl-SN-glycero-3-phosphoethanolamine} were used to deliver non-fluorescent DNA. Fluorescent lipid delivery was also observed to be nearly 100% in 2 $CFSME_{0}$-, HeLa and Hep 2 g cells when used in a transfection protocol similar to that described in Figures 1 and 2.

II.A.3. Gene Transfer of a Nuclear Targeted Lac Z Gene to Rat Airways in vivo.

Distribution of in vivo gene expression in the rat lung was evaluated following direct installation of DOTMA/DOPE/DNA conjugates using a nuclear targeted Lac Z construct. Forty-eight hours following Lac Z gene transfer, two intact rat lungs were isolated and lavaged in situ and then removed en bloc and stained for Lac Z activity using a method described by Yoshimura, et al (14). Using this protocol, X-gal staining resulted in a predominantly bilateral perihilar pattern of reporter gene expression (Fig. 4), suggesting ß-galactosidase activity in the large and medium-sized airways. No perihilar staining was observed in paired controls transfected with a plasmid containing the human CFTR cDNA. The histologic distribution of reporter gene expression was evaluated by X-gal staining of lung frozen sections. Using DOTMA/DOPE, 7 of 8 Lac Z treated rat lungs demonstrated nuclear Lac Z reporter gene activity as shown in Figure 5. Over 60% of bronchioles in these animals showed evidence of reporter gene expression, with up to 80% of cells positive per airway. None of eight matched control animals treated with DOTMA/DOPE/CFTR conjugates or lipid alone showed significant staining, (i.e., $<1\%$ of cells positive). Similar results were obtained in eight animals studied with DMRIE/DOPE.

In addition, approximately 0.5-1% of alveolar Type II cells isolated from a rat treated in vivo with DOTMA/DOPE/nuclear targeted Lac Z showed evidence of reporter gene activity when isolated 72 hours following gene transfer and studied after four additional days in culture (Fig. 5). This result indicates that cationic liposomes can mediate gene expression in alveolar cells following direct installation, although to a much lesser degree than in the large and medium-sized airways.

II.A.4. In vivo Toxicity of Lipid DNA Conjugates.

In order to evaluate the acute toxicity of lipid mediated gene transfer in the rat lung,

plastic embedded lung tissue was prepared and studied 72 hours following DOTMA/DOPE administration of plasmids containing either CFTR or E. coli Lac Z. Sections from animals were stained with either hemotoxylin and eosin or methylene blue; these sections revealed essentially normal airway and airspace histology with negligible inflammation. An occasional, mild mononuclear cell infiltrate such as that shown in Figure 6 was observed in <5% of airways, and appeared to be the result of lipid/DNA administration. In addition, although the in vivo rat lung experiments were designed to deliver lipid/DNA conjugates in a highly effective manner, because of the small size of the animals used, and the requirement for obtaining large cross-sectional tissue samples exhibiting high level expression of reporter genes or CFTR (e.g., large segments of efficiently transfected trachea for mounting in Ussing Chambers, see below) the in vivo approach used in our preclinical studies was necessarily traumatic. Two post-operative deaths were observed in 42 animals which underwent anesthesia and surgery and were directly attributable to technical aspects of the procedure (i.e., anesthesia related deaths due to difficulty titering appropriate levels of inhaled general anesthesia in the setting of respiratory depression during instillation of a very large volume [approximately 1/10th total lung capacity] of lipid/DNA complex solution). The human protocol eliminates all such risks associated with anesthesia and surgical administration of lipid/DNA conjugates (i.e., no inhaled anesthesia, no surgical dissection, no cannulation of the trachea, and very small relative volumes of administered lipid DNA/conjugates, as described in Section III, below). If nasal administration as described in this protocol leads to appropriate correction of the CF defect, after the development of additional preliminary data, a modified version of our animal protocol for CF lung administration would be proposed. Such an approach, for example, could involve non-surgical, non-anesthesia-dependent administration (i.e., with very small volumes of lipid/DNA conjugates delivered to specific lung segments via a bronchoscope) or aerosolized administration of lipid/CFTR cDNA. Prior to administration of lipid/CFTR conjugates (by aerosolization, instillation, or any other mechanism) to the lower airways of CF lungs, therefore, a conservative first step in the interest of maximizing patient safety is topical administration to nasal airway epithelium; this approach is the one proposed in the current protocol (see study methodology, Section III). As with the present protocol, all human experiments will be performed after full approval of the FDA regarding both safety and efficacy.

II.A.5. Expression of Functional CFTR in Rat Trachea

The tracheas of 19 animals treated with DOTMA/DOPE and plasmids containing human CFTR or nuclear targeted Lac Z were studied in Ussing Chambers for evidence of human CFTR chloride channel activity. Of the seven Lac Z transfected tracheas, none had a measurable response to forskolin, indicating very low levels of endogenous rat CFTR activity (Fig. 7 and 8). However, in ten of twelve CFTR treated tracheas, forskolin produced a small, rapid increase in the I_{sc} (approximately $7.0 \pm 2.0 \mu A$ per cm^2)($p < .03$). Bumetanide (which blocks the basolateral Na^+-K^+-$2Cl^-$ cotransporter) reversed the forskolin effect (a result compatible with overexpression of endogenous CFTR), and produced a further inhibition of the I_{sc} in the CFTR transfected tracheas. In a slight modification of the above protocol, designed to enhance detection of endogenous CFTR, indocin was used to block prostaglandin induced basal Cl^- secretory activity to augment the response to forskolin. In three tracheas treated with the Lac Z gene (complexed with either DOTMA/DOPE or DMRIE/DOPE) the mean I_{sc} increase following forskolin treatment was $2.11 \mu A/cm^2 \pm 0.71$, while in 12 tracheas treated with CFTR cDNA, the mean

increase in short-circuit current induced by forskolin was 11.04, ±5.17. These results indicate that lipid mediated gene transfer successfully introduced a cyclic AMP dependent chloride secretion that was barely detectable in control tissues. An increase in cAMP dependent current is the anticipated result of human CFTR expression in a tissue with otherwise minimal endogenous forskolin stimulable chloride secretion.

These results are among the first to demonstrate transfer of an active human CFTR to an intact mammalian epithelium in vivo. Previous reports have demonstrated correction of the $Cl^{(-)}$ transport defect in the trachea of transgenic (cf/cf) mice using DOTMA/DOPE/DNA complexes. However, 1) the presence of an endogenous cAMP stimulated Cl^- channel (distinct from CFTR within the airways of these mice and 2) the young age and severe gastrointestinal manifestations (including malnutrition) of the CF animals limit the use of the CF mouse in gene transfer based experiments targeting the pulmonary epithelium. The rat trachea may represent an alternative model for studies of CFTR gene transfer in this context, as suggested by efficient reporter gene transfer and relatively low activity of the endogenous rat CFTR protein. The discrepancy between in vitro and in vivo transfection efficiency (Figures 2 and 5) may relate to differences between the cell types studied, the conditions used, or to the poorly understood mechanisms within cells which allow the expression of plasmid DNA based genes under the regulation of eukaryotic promoters. We have observed that although only 5% of human respiratory epithelial cells express reporter genes when driven by the SV40 early promoter in vitro (Fig. 2), virtually 100% of these cells receive the lipid/DNA conjugate and rapidly transport the exogenous DNA to the cell nucleus (Fig. 3). We also have recently demonstrated that promoters of increasing strength not only lead to enhanced overall signal, but to an increased proportion of cells expressing Lac Z (10% using the SV40 promoter vs >90% using a Vaccinia/Lac operon plasmid-based promoter) (data not shown). These results indicate that delivery of plasmid DNA to respiratory epithelial cells in culture is much more efficient than the fraction of cells which are able to express the DNA at detectable levels, and implicates features inherent in the cells (rather than the particular lipid formulations) as contributing to the observed efficiency of lipid based gene expression. Such cell-specific factors might contribute to differences in the efficiency of gene transfer observed in vitro compared with cellular expression in vivo.

These results represent the first in vivo demonstration of cationic lipid gene transfer to the respiratory epithelium of animals other than mice using a lipid formulation other than lipofectin. The finding of successful gene transfer using cationic liposomes in a second animal species together with high activity in CF and normal human respiratory epithelium in vitro increases the probability that the results can be extrapolated to human subjects. Considering this information, efforts aimed to develop human based trials of lipid DNA conjugates to correct the CF defect in airways of patients with CF now seem appropriate. Human based trials will be required in order to evaluate both the efficacy of CFTR gene transfer and the possible safety or toxicity of this approach in vivo.

III. METHODOLOGY

III.A. Introduction:

The pre-clinical data and background information provided above indicate that lipid mediated CFTR transfer to respiratory epithelium may constitute an important alternative to viral-based DNA delivery. The overall design of our Human Protocol will be a non-randomized,

non-blinded, ascending dose administration of lipid/CFTR cDNA conjugates to nasal airway epithelium, similar in terms of target site to protocols previously approved by the Recombinant DNA Advisory Committee for the evaluation of recombinant adenovirus mediated CFTR delivery (Welsh and Smith, RAC Protocol #9212-036; Boucher and Knowles, RAC Protocol #9303-042). For each patient in the study, cationic liposomes will be used to deliver CFTR to one nostril, and a mock transfection (lipid/DNA conjugate lacking CFTR) of the contralateral nostril will be performed as a negative control. Three ascending dosages of CFTR cDNA will be utilized. The patients will not be paid for their participation in the study nor will a patient questionnaire or interview be utilized.

III.B. Specific Aims of the Human Protocol

1. Evaluate safety of lipid-mediated gene transfer to nasal epithelium of CF patients. Local inflammation and injury of the mucosal and sub-mucosal tissues will be evaluated following gene transfer, both in CFTR and (contralateral) mock transfected nasal epithelium. Systemic responses to the administered lipid/DNA conjugate (blood counts, serum chemistries, serologic antibody responses to the lipid or DNA) will also be addressed.

2. Evaluate efficacy of wild-type CFTR cDNA administration in CF nasal epithelium in vivo. Three ascending dosages of CFTR cDNA will be evaluated. Nasal respiratory epithelium will be studied for expression of CFTR protein and mRNA, for correction of the ion transport abnormality associated with the disease within nasal epithelium, and for the duration of these responses.

III.C. Use of Nasal Epithelium for Recombinant Gene Administration

Airway epithelium immediately below the inferior turbinate will serve as the target for gene transfer in these experiments. This epithelium is histologically similar to the epithelium of lower airways, and is readily accessible for studies of the effects of lipid-mediated gene transfer. CF airway epithelium within the nose 1) exhibits a well-characterized ion transport abnormality which parallels that observed in lower airway epithelium, 2) can be used to directly evaluate electrical correction of the CF defect following gene transfer and 3) allows studies of gene transfer without the complicating features of chronic, severe inflammation and scarring which characterize the lower airways. Additionally, by performing initial gene transfer in the nasal epithelium, the risks associated with repeated bronchoscopy (both for gene delivery and for evaluation following gene transfer) will not be included in this Phase I trial.

III.D. Patient Evaluation

Prior to and during the gene transfer protocol, a series of tests will be undertaken in order to establish baseline information relevant to the patient's clinical status (severity of CF and non-CF related illnesses) and the magnitude of the transport abnormalities within the nasal epithelium of study participants. These tests include:

1. Complete history and physical examination, blood count and differential, blood chemistry evaluation including tests of liver function (transaminases, LDH, bilirubin), renal function (BUN, creatinine), serum electrolyte levels, and whole blood for CFTR genotype analysis.

2. EKG (12 lead), urinalysis.
3. Chest x-ray (PA and lateral), arterial blood gases obtained on room air, oximetry, sinus films, pulmonary function tests (including spirometry, lung capacity measurements, and diffusion capacity), and sputum culture.
4. Nasal endoscopy, nasal washes for culture, nasal brushings and biopsy and for histopathology.
5. Sperm count for males not already known to be azoospermic; pregnancy test for females prior to entry into the study.
6. Nasal transepithelial potential difference measurements.
7. Serum sample to measure baseline antibody titers against lipid/DNA conjugates.

III.E. Clinical Protocol

1. Study Design. This is a non-randomized, non-blinded Phase I trial of Lipid/DNA conjugates for use in gene transfer of CFTR to respiratory nasal epithelium.
2. Pre-treatment evaluation. Patients will enter the study and written, informed consent will be obtained prior to participation in the screening evaluation. Pre-treatment evaluation will be performed 4 weeks prior to gene transfer and at the time of admission to the Clinical Research Center (Day 1, see below) and will include:
   a. Complete history, chart review.
   b. Physical examination.
   c. Serum chemistry evaluation (including serum alkaline phosphatase, bilirubin, LDH, BUN, creatinine, albumin, calcium, phosphate, sodium, potassium, chloride, CO_2, glucose measurements) and complete blood count (including platelets and differential), urinalysis, as well as CF genotype analysis.
   d. Sperm count on males not previously known to be azoospermic; pregnancy test on females.
   e. Nasal endoscopy.
   f. Serum for analysis of antibody to DNA/lipid conjugates (baseline studies).
   g. Pulmonary function tests, including diffusion capacity.
   h. Arterial blood gases and oximetry.
   i. 12-lead EKG.
   j. PA and lateral chest x-ray. Nasal/sinus x-rays.
   k. Sputum culture and antibiotic sensitivity.
   l. Measurement of nasal potential difference.
   m. Nasal brushings/washings.

In addition, at 14d and 1d prior to gene transfer, patients will undergo history and physical examinations, oximetry, nasal potential difference measurements, and serum sampling for antibody to DNA/lipid conjugates (baseline studies).

3. Gene transfer procedures.
   Patients will be admitted to a private room in the Clinical Research Center at The Children's Hospital of Alabama on the day of gene transfer. The patient will be placed in a left lateral decubitus position and given mild sedation (Midazolam, 2-3 mgs IM). Nasal endoscopy (i.e., flexible or short rigid [Hopkins rod] examination) will be used to

define a 3-5 mm region along the inferior surface of the right inferior turbinate suitable for lipid/DNA conjugate administration. This area will be gently rinsed with normal saline prior to gene administration. Under direct visualization, a 1 ml volume of lipid/DNA formulation (see below) will be administered using a syringe pump, over a 30 minute period. The patient will then be turned to a right lateral decubitus position and a nasal endoscopy used to identify a corresponding contralateral region beneath the left inferior turbinate. A 1 ml volume of lipid/DNA solution without CFTR will then be administered over 30 minutes.

4. Lipid/DNA formulations. The lipid formulation which will be used in this study is a 1:1 molar mixture of DMRIE with DOPE. The DMRIE/DOPE and DNA used in this clinical protocol will be generated at Genzyme, Inc., (Framingham, Mass.) under GMP guidelines and following FDA-approval and supervision. Lipid and plasmid DNA will be packaged desiccated in separate vials and will arrive at the University of Alabama at Birmingham by overnight carrier. The vials will each be hydrated with sterile, distilled water in a laminar flow hood located in the IV Room of the Pharmacy, Ground Floor of The Children's Hospital; this is the usual location of mixing of intravenous solutions for patient administration. In vitro evidence suggests that gene transfer using lipid DNA conjugates requires optimization of the lipid:DNA base ratio. We will study three patient groups (see Table 1, Appendix); each group with a different ratio of CFTR plasmid DNA:lipid formulation. CFTR plasmid DNA used in these experiments will be the pKCTR, which contains CFTR cDNA under regulatory control of the SV-40 early promoter (Figure 7, Preliminary Data Section). Mock (no CFTR) transfections will be performed using a vector derived from pKCTR in which CFTR, as well as SV-40 promoter and polyadenylation sequences have been deleted.

   Based upon experiments using similar proportions of lipid:DNA in vitro, and because the range used in this study includes both lipid excess and DNA base excess, this range offers the best likelihood of encompassing the appropriate proportion of lipid:DNA for optimal gene transfer. Assuming that 1 cm^2 of nasal mucosa has approximately $2x10^6$ cells along the surface epithelium and that the nasal mucosa to be studied will contain on the order of 5 times this area (an estimated 10^7 cells) even at the lowest dose of DNA (100 mcg) approximately $3x10^6$ copies of CFTR plasmid DNA will be administered per target cell.

5. Evaluation following lipid/DNA conjugate administration.
   Patients will be monitored in the clinical research center with vital signs every 30 minutes for the first two hours following gene administration, then on an hourly basis for the next six hours, every two hours for the next 16 hours and finally every 6 hours for the duration of the admission to the CRC. Admission to the CRC will be for a total of five days. During hospitalization all standard cystic fibrosis therapies will continue. In order to maximize safety, each patient's hospitalization will be completed prior to proceeding to the next patient. A description of the procedures to be used is given below, followed by a flow sheet schedule (see Table 2, Appendix) of the specific tests to be used in this protocol.

a. Toxicity.

1) Serial assessment by history and physical examination, and measurements including blood chemistry, complete blood count, pulmonary function tests, oximetry, EKG, chest x-ray and sputum culture will be performed in order to evaluate individuals in this study for evidence of local (nasal), pulmonary or systemic toxicity. Serum will also be studied for evidence of humoral immune responses to administered lipid or DNA. [1]

2) Direct visualization of nasal mucosa for evaluation of inflammation or other changes will be accomplished using nasal endoscopy. This procedure will be performed in collaboration with Dr. Brian Wiatrak (Department of Pediatric Otolaryngology). After local anesthesia with 2% Xylocaine a flexible nasal endoscope will be used to visually observe the inferior turbinate area for evidence of edema, exudate, or purulence following lipid or lipid/DNA administration.

3) Inflammation at the site of CFTR gene administration and mock (no CFTR) administration (in the contralateral nostril) will be studied using nasal biopsy, nasal brushings and washings,as described below:

aa. Nasal biopsy. Biopsies of nasal epithelium will be performed in order to evaluate inflammation and other cytopathic effects due to lipid/DNA conjugate administration. This procedure will be performed by Dr. B. Wiatrak as follows: The nasal cavity will initially be decongested with 2% Pontocaine/.25% Phenylephrine on cotton pledgets. The site of the biopsy will be the inferior aspect of the inferior turbinate with an area of approximately 0.5cm^2. The area will be injected submucosally with 1% Lidocaine with 1:100,000 Epinephrine. The mucosal biopsy will then be excised using a #11 scalpel blade and hemostasis obtained by placing 25% Neo-Synephrine-soaked pledgets. Biopsies will be assessed for evidence of mucosal disruption, cytolysis, or inflammation using light microscopy. Electromicroscopy will also be performed for evidence of cytopathic effects. Tylenol will be given as required for analgesia.

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1. Although lipid and DNA are generally poorly immunogenic, in order to determine whether a systemic humoral antibody response develops in animals given lipid/DNA conjugates, a series of experiments will be performed in which rats given 500μg of plasmid DNA conjugated to 400μg DMRIE/DOPE in a final volume of 800μl (as in Figure 4, Preclinical Data Section). Rats will be bled at 1,2,3, and 4 weeks following intratracheal administration. Serum from these bleeds (as well as from a pre-DNA administration bleed) will be used in an ELISA assay in which 1μg samples of lipid/DNA conjugates per microtiter well are overlaid with dilutions of serum between 1:10 and 1:10^7. Goat anti-rat IgG conjugated to alkaline phosphatase will be used to detect any antibody arising from lipid/DNA conjugates using a standard ELISA protocol (for example, see Sorscher, et al, *Am J Physiol (Cell Physiol)* 255:c835-c843, 1988). Similar experiments (using an antihuman IgG conjugated to alkaline phosphatase) will be performed as part of the human study described here.

bb. Nasal brushings and washings. Nasal mucosa will be pre-treated with 0.05% oxymetazoline hydrochloride (Afrin) and topically anesthetized with 2% Lidocaine five minutes before brushing. Using a Pap smear cytology brush, the nasal mucosa at the site of gene administration below the inferior turbinate will be brushed gently to remove non-adherent cells. Cells recovered in the brushings will be disassociated in phosphate-buffered saline. Cystospin of these cells, as well of cells washed from the nasal mucosa with general application of a moistened cotton-tipped pledget will be analyzed for cell count, morphology, and cytopathic effects.

b. Efficiency of gene transfer.

1) Determination of gene transfer efficiency will be based predominately on measurements of the nasal potential difference. When ions move across the nasal mucosa, ion currents (and therefore potential differences) are generated. This potential difference is known to be significantly elevated in CF patients compared with non-CF controls. This well-characterized electrical abnormality of the CF nasal airway also reflects the abnormalities observed in the trachea and lower airways of CF patients. In order to evaluate nasal potential difference, a reference electrode (a 21-gauge butterfly needle filled with a 4% agar/Ringer's lactate solution) is inserted subcutaneously in the volar aspect of the forearm. The reference bridge is connected through a calomel cell in 3M KCL to a voltmeter (BK Precision Instruments, Model 2906). An exploring bridge is established by inserting a second agar/Ringer's lactate solution-filled needle into a PE 50 tubing line through which normal saline is being perfused at the rate of .2 cc per minute. The exploring bridge needle is connected through a calomel cell in 3M KCL to the volt meter. Nasal potential difference is measured by gently placing the tip of the exploring PE tubing line against the nasal mucosa, and can be used to evaluate different areas within the inferior turbinate, the septum, or elsewhere within the nose. Because the nasal PD is measured essentially instantaneously, 15-20 measurements at different points in both the experimental and control sides of the nose can be generated in a relatively short (10-15 minute) period. Measurements within the area of lipid/CFTR gene and control (no CFTR) administration, as well as other regions within the nose not receiving gene (e.g., septum, lateral wall) will be studied in both nares. The nasal potential difference can be performed repeatedly without damage to the nasal mucosa. Dr. Sorscher has previously performed this test on 10 CF patients and 15 non-CF controls without complications, and with verification of elevated potential difference in the CF patients. Correction of this elevated potential difference following gene administration (without change in the patient's opposite nostril which receives no CFTR gene) would serve as evidence for gene transfer of the active CFTR. Nasal PD measurements within a given CF patient are quite stable; for example, daily PD measurements over a two-week period on one CF patient performed by Dr. Sorscher showed no statistically significant differences. Nasal PD measurements are currently being used as part of a study preliminary to CFTR gene transfer at The Children's Hospital of Alabama, Division of Pulmonary Medicine. amiloride

(100 micromolar), a drug known to block the elevated potential difference present in CF (and to bring the PD into a range similar to that observed in normal nasal epithelium following amiloride treatment) and terbutaline (100 micromolar) which stimulates normal CFTR will be superperfused onto the nasal mucosa in order to quantitate the proportion of nasal PD in patients attributable to amiloride or terbutaline sensitive transport.

2) Efficacy of CFTR gene transfer will also be evaluated using nasal biopsy, brushings, and washings obtained following administration of lipid/DNA conjugates. CFTR protein production and localization at the apical membrane will be studied using immunocytochemistry and antibodies raised against CFTR. The goal in antibody localization experiments will be to determine whether an apically localized CFTR can be observed specifically in biopsies following wild-type CFTR administration. As noted above, for the Delta F508 CFTR homozygotes studied in this protocol, no apically targeted CFTR is anticipated since the mutation leads to maturational arrest of the mutant protein within the endoplasmic reticulum. Our laboratories have previously used CFTR antibodies to localize the CFTR protein to the apical membranes of cell sections (see Morris, et. al., included in appendix). In addition, localization of CFTR to apical membranes of respiratory tissues has been reported previously by Dr. John Cohn (Duke University) using affinity purified antibody to the carboxyl terminal of CFTR. Dr. Cohn is currently collaborating with our group in localization experiments. Finally, we have recently developed a polyclonal antibody to the CFTR first nucleotide binding domain. In preliminary studies, this antibody recognizes the apical CFTR protein in tissues of normal mice, but not in mice in which the CFTR has been deleted. All these reagents will be utilized to optimize the chances of observing normal human CFTR expression in the apical membranes of human nasal epithelial cells transfected with the normal CFTR gene.

Nasal epithelial cells recovered in biopsy brushings and washings will also be used to test for the presence of CFTR mRNA by reverse transcription PCR techniques. The approach for reverse transcribed PCR based detection of human CFTR has been used previously both for detection of human CFTR expression following gene transfer to murine lungs using cationic liposomes (14) and to detect transferred CFTR in very small numbers of cells obtained following bronchoscopic brushing in human patients (49). This technique will permit a highly sensitive measurement of normal CFTR expression in vivo, and coupled with PCR based detection of CFTR wild-type and mutant mRNA's (Sorscher and Huang, *Lancet*, 337:1115-1118, 1991) will allow confirmation of wild-type CFTR in biopsy specimens taken from study patients.

III.F. ALTERNATIVE TREATMENTS

Standard cystic fibrosis therapy (chest physiotherapy, nutritional measures, antibiotics) as well as experimental therapies (rh DNase) are also available for the treatment of CF. If a patient decides not to participate in this study, it will not effect his/her care at the University of

Alabama at Birmingham, and he/she will continue to receive the best available standard treatment for CF. The patient agrees that by participation in this study, he/she will not enter into another experimental study (for example, a rh DNase trial) at the same time. All standard CF therapy will remain available to the patient during his/her participation in this study. A decision not participate or to withdraw from the study will not effect a patient's care at the University of Alabama at Birmingham, and he/she will continue to receive the best available standard treatment for CF.

III.G. SUMMARY OF CLINICAL TRIAL

The studies described here will provide information concerning the response in humans to application of lipid/DNA conjugates to a limited region of the nasal mucosa. The potential sequelae of administration, including inflammation or cytopathic effects at the site of gene delivery, as well as more distant effects such as humoral antibody responses to vector and DNA, as well as the duration of these responses, will be evaluated. Results from this study will provide useful information concerning cationic liposome-mediated delivery of ion transport competent CFTR, evaluation of CFTR protein and mRNA expression at the target site of gene transfer, information about optimal lipid:DNA ratios for use in human respiratory mucosal gene transfer using cationic liposomes, and the possible duration of CFTR expression after plasmid based DNA administration in human nasal mucosa. Because nasal respiratory epithelium is a well-established model for study of the CF ion transport abnormality which exists in the lower airways, results obtained in this protocol will be directly relevant to future therapies aimed at correcting the genetic defect which leads to CF. If safe, functional CFTR expression can be shown in nasal respiratory epithelium using cationic liposome-mediated gene transfer, this study will set the stage for administration of lipid/CFTR gene conjugates to the lower airways and possible amelioration of the progressive respiratory damage caused by mutant CFTR. Any information obtained in this study concerning the duration of functional CFTR effects on CFTR mRNA on protein expression or nasal bioelectric correction will also be significant, since this data will suggest an appropriate course for a future study of repeated CFTR gene administration within the nasal airway. On the other hand, substantial local or other toxicity will indicate the need to explore other cationic lipid formulations, both in vitro and in vivo, as well as alternate vector systems for gene delivery. By focusing this study on nasal rather than lower airway epithelium, the study will aim to evaluate important initial questions while placing the CF patients involved in the protocol at a minimum of risk. The results obtained from this study are highly likely to provide useful information concerning lipid mediated gene transfer as a potential therapy for cystic fibrosis.

III.H. OVERALL ASSESSMENT OF RISKS AND BENEFITS IN THIS STUDY

The risks involved in this study relate to nasal administration of lipid/DNA conjugates and the procedures designed to test safety and efficacy of gene delivery.

a. Risks associated with DNA administration to nasal respiratory epithelium include local pain or irritation, and the discomfort associated with approximately one hour of lying very still in a lateral decubitus position. Attempts will be made to minimize these discomforts by use of topical anesthesia and mild oral sedation. The nasal mucosal target for gene delivery is at risk of local inflammation and/or cytopathic effects mediated by lipid/DNA conjugates. Although cationic liposomes are clearly toxic in vitro at high

doses, broad ranges can usually be defined for most cell types in which efficient gene transfer occurs with minimal cytotoxicity. Our in vivo studies, as well as studies from other laboratories, have failed to show significant local respiratory mucosal toxicity over the range of lipid/DNA conjugate concentrations which will be used in this protocol. The risks also include long term scaring of the mucosa beneath the inferior turbinate, although the risk of chronic scarring occurring after a single administration seems very small. Humoral immune response to lipid/DNA conjugates could occur, and it is possible that immune response against liposomes containing DNA might limit repeated administration of the CFTR. Because DNA in large amounts is constituitively present within the lung (and appears to contribute to CF sputum viscosity) it seems less likely that a single administration of plasmid DNA within the nose will lead to a clinically significant or limiting immune response. In addition, lipid carrier molecules (for use in delivery of drugs, recombinant proteins, etc.,) in general have not been highly antigenic in human or other in vivo studies. Nevertheless, possible systemic response to localized nasal administration of lipid/DNA conjugates (including antibody generation to these conjugates), will be monitored during this study. The characteristics of any such responses will be carefully documented. Because some evidence suggests that the Delta F508 CFTR may not appropriately target to the apical cell membrane, it is possible that over-expression of a wild-type CFTR on the cell surface could evoke antibodies directed against the CF protein itself. However, 1) cellular synthesis without membrane targeting does not preclude immune tolerance and 2) experiments in transgenic mice in which human CFTR was over-expressed in respiratory mucosa failed to show any adverse effects, suggesting that expression in an animal naive to wild-type human CFTR did not evoke a significant anti-CFTR immune response. Finally there is 1) a theoretical risk of integration of plasmid within the host genome, resulting in malignant transformation of the host cell, and 2) development of replication competence by transfected plasmid DNA. In general, these risks are much smaller than those observed with the viral based DNA delivery systems (e.g., retroviral, adenoviral). Such events have never been observed using lipid/DNA conjugates as reviewed by Nabel (55).

b. Risks associated with procedures to test safety and efficacy.

1. Blood drawing. Venipuncture and arterial sampling (for blood gases) can produce modest discomfort and bruising as well as vasovagal symptoms. Local anesthesia (with Xylocaine) will be used during arterial blood gas sampling if requested. Risk and damage to the vascular system of the hand after radial artery puncture is very small and can be minimized further by assessing collateral circulation to the palm using an Allen test. These procedures are without substantial long term risk.

2. Measurement of nasal potential difference. This is associated with minimal discomfort at the site of the reference needle placed subcutaneously in the forearm. Potential risks include bruising, bleeding, and infection, but these have never been observed at the reference needle site in over 50 individual measurements performed by Dr. Sorscher. No cutaneous infection has been observed in over 10 years of experience as reported by the group which developed the nasal potential difference protocol at the University of North Carolina, Chapel Hill. Perfusion of Ringer's lactate onto the nasal mucosa is usually very well tolerated

with minimal irritation but can be associated with sneezing or tearing.

3. Nasal brushings and washings. These are moderately uncomfortable and will be performed after local anesthesia is provided. Pain, and the small risk of bleeding following the procedure, can be managed by applying gentle pressure directly to the site. Gentle swabs of the nasal mucosa are associated with minimal, if any, discomfort.
4. Nasal endoscopy. Nasal endoscopy is a standard ENT procedure which involves modest discomfort, usually well-managed with topical anesthesia and mild sedatives. The small risk of bleeding following the procedure exists; bleeding can be managed by direct pressure to the site with a cotton-tipped applicator or gauze.
5. Nasal biopsy. Bilateral nasal biopsies are planned for each CF patient enrolled in this study (in order to exclude significant inflammation at the site of gene administration and to screen for wild-type CFTR expression at mRNA and protein levels). Biopsy of the nasal epithelium produces moderate pain and discomfort, and local anesthesia as well as oral anesthesia, if necessary, will be used to minimize these symptoms. There is also a small risk of bleeding and infection at the biopsy site, either of which can be generally addressed by appropriate treatment, e.g., applying pressure to the bleeding site under direct visualization; use of antibiotics to control infection. A small risk of associated scar formation at the biopsy site is also present.
6. 12-lead EKG, sputum culture, and pulmonary function tests are routinely performed on CF patients and no significant risks from these tests are anticipated.
7. Chest and sinus x-rays carry the risks associated with radiation exposure. Although no direct adverse affects attributable to the low doses of radiation used for the tests in this study have been demonstrated, the cumulative effects of radiation over a patient's lifetime (particularly in the setting of multiple chest x-rays and other radiographic studies which CF patients undergo) is not known.
8. Emotional discomfort attributable to a 5-day hospitalization may be significant. Television, reading materials, social service support, VCR, video games, etc., will be made available to patients during their hospitalization in the Clinical Research Center.

c. Risks to hospital or other personnel. The risks for spread of lipid/DNA conjugates to hospital personnel and others is very low. There is no evidence that DNA prepared and transferred to eukayotic cells in the manner used in this study is capable of replication or independent gene transfer. However, because aerosolization of plasmid DNA complexed to lipid could occur during or immediately following the time of administration into the patient's nose (e.g., as a result of the patient sneezing) and since lipid DNA conjugates have been shown to mediate efficient gene transfer into murine lungs following aerosolization, respiratory precautions will be taken by all personnel during the first day of the patient's admission to the CRC.

Overall Risk-Benefit Ratio

This is a Phase I study of administration of the CFTR gene to a very limited area of the respiratory epithelium. Because it is not anticipated that correction of the CF electrolyte transport abnormality within a small segment of the nasal airway will lead to any improvement

in clinical status of patients involved in this study, participants in this trial will not benefit directly. However, because the nasal airway functions similarly to lower airways, and exhibits the CF transport defect, safe, gene-transfer mediated correction of the ion transport defect within the nasal airway would establish the feasibility of a trial of lipid mediated gene transfer within the lungs of CF patients. Because lung disease represents the predominant cause of death in patients with CF, the information gathered in this study is therefore very likely to benefit the CF community as a whole. Most patients with CF die before age 30 despite the best available therapies. Adenovirus-based delivery of CFTR is still in the experimental stages and little is known either about safety or efficacy in human trials. Since five independent laboratories have now reported safe and efficient gene transfer of CFTR or other reporter genes to non-human, mammalian respiratory epithelium using cationic liposomes, it is reasonable to evaluate this potential avenue for gene delivery in a limited Phase I trial in humans. The information gained from this study will add to our understanding of lipid based gene delivery and will serve the critical need of developing new and better therapies for CF lung disease.

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III.E. APPENDICES
TABLE 3

Inclusion Criteria

a. Diagnosis of CF based upon sweat chloride level greater than 60 mEq/l together with clinical manifestations of CF.
b. Male and/or female patients greater than 18 years of age. Males entered into the study must have documented azoospermia. More than 95% of CF males are infertile and have azoospermia associated with congenital vas deferens atrophy.
c. Mild to moderate pulmonary disease (FEV_1 greater than 40% predicted) and no pulmonary exacerbation within the four weeks prior to entry into the study.
d. Genotype: Because of likely heterogeneity in the molecular pathogenesis of CF, homozygous Delta F508 patients will be preferred. Other CF patients will be eligible if they both 1) are compound heterozygotes for the F 508 mutation and 2) have pancreatic insufficiency.

Exclusion Criteria:

a. Patients demonstrating evidence of sequelae of CF which might make participation in this study difficult or of higher risk will be excluded.
    1). Severe cardiac, hepatic or other organ system disease, including juvenile-type (insulin-dependent) diabetes mellitus.
    2) Acute or recurrent sinusitis or history of obstructive nasal polyposis.
    3) PO_2 less than 60 mm mercury assessed by room air blood gas.
    4) Women not practicing birth control for at least one month prior to admission into the study, or with a positive pregnancy test during the pre-study evaluation.
    5) Unstable lung disease, with worsening pulmonary symptoms, arterial blood gases, or pulmonary function tests within the two weeks prior to entry into the study.
    6) Corticosteroid therapy within the previous four months.
    7) Pneumothorax or marked hemoptysis (e.g., greater than 250 cc of blood within a 24-hour period) within the past year.
    8) Inability in the opinion of the investigators to comply with or comprehend the protocol.
b. DNase use or participation in another experimental clinical study within the past two months.

INFORMED CONSENT
University of Alabama at Birmingham
The Children's Hospital of Alabama
Birmingham, Alabama

Informed Consent

INFORMED CONSENT TO PARTICIPATE IN A RESEARCH STUDY

GENE THERAPY FOR CYSTIC FIBROSIS USING CATIONIC LIPOSOME MEDIATED GENE TRANSFER: A Phase I Trial of Safety and Efficacy in the Nasal Airway

Principal Investigators:

Eric J. Sorscher, M.D.
James J. Logan, M.D.

Co-Investigators:

Raymond A. Frizzell, Ph.D.
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P.L. Felgner, Ph.D.
Sadis Matalon, Ph.D.
Lynn Walker, M.D.
Brian J. Wiatrak, M.D.

INFORMED CONSENT

I have been asked to take part in a Research Study. Before agreeing to participate, it is important that I read and understand the following explanation. I understand that I will be one of about nine (9) subjects in this study.

PURPOSE

Human genes carry functions which are necessary for healthy human life. Cystic fibrosis (CF) is caused when one of these genes, called the cystic fibrosis gene, cannot function properly. As a result of this dysfunction, tissues in the bodies of CF patients (and the cells which are the building blocks of these tissues) cannot move salts properly. For example, the high content of salt in the sweat of CF patients, and the thick respiratory mucous in the lungs of CF patients, have both been associated with difficulties moving salts such as sodium and chloride.

The purpose of this study is to determine whether normal, functional copies of the CF gene can be delivered to the cells lining the nose of CF patients. This study will use small amounts of molecules of fat (also called "lipids") to carry the normal gene into these cells.

This study has two goals. The first goal is to determine whether lipids can be used to safely carry the normal gene into the cells lining the nose. The second goal is to determine whether after receiving the normal CF gene, the inside of the nose will develop an improved ability to move salts.

The use of genes to treat human diseases has been called "gene therapy". Because the serious complications of CF occur in parts of the body other than the nose (for example, in the lungs and pancreas), it is unlikely that the delivery of a normal CF gene to the inside of the nose will make participants in this study healthier. On the other hand, the overall goal of this research study will be to allow the possibility of attempting the same type of normal gene delivery to CF lungs, with the hope of developing better treatments for cystic fibrosis.

DURATION

I understand that I will participate in this study for approximately five (5) months. I will be evaluated in the Cystic Fibrosis Clinic at The Children's Hospital of Alabama at 28 days, 14 days and 1 day prior to receiving the CF gene. Each of these evaluations will take approximately three (3) hours. On the day the gene is given, I will be admitted to the Clinical Research Center at The Children's Hospital of Alabama and will remain in the hospital for a period of approximately five (5) days.

After this period, if there are no other significant medical problems, I will be discharged and followed on a daily basis in the Outpatient Clinic for one week (7 days), then twice a week for the following three weeks, and then every 2-3 weeks for the next 10 weeks. After this period, I will keep my regular Cystic Fibrosis Clinic appointments and be seen approximately

every three (3) months.

PROCEDURES WHICH WILL BE PERFORMED AS PART OF THE RESEARCH STUDY

If I agree to participate in this study, the following procedures will be performed:

1. A complete physical examination, including chest x-ray, sinus x-rays and pulmonary function tests with arterial blood gases will be performed prior to the administration of the normal CF gene. Pulmonary function tests and blood gases will also be performed once following administration of the normal CF gene. X-rays will also be repeated after the gene is given.
2. Blood samples (approximately 2 tsp each) will be taken 10-12 times over the five-month period of the study. The type of defect in my CF gene will be evaluated. These tests will also evaluate the function of vital organs (for example liver and kidneys) and blood counts. Sputum cultures, oximetries, urinalyses, and electrocardiogram will also be performed as part of this study. A pregnancy test (females) or sperm count (males) may also be performed.
3. Administration of the normal CF gene. This will be accomplished in a hospital bed in The Children's Hospital Clinical Research Center. On the morning of gene administration, I will be asked to lie on my side and a small flexible tube (about the thickness of a kite string) will be placed 2-4" inside one nostril. About 1/2 tsp of a solution containing lipid and a normal CF gene will be very slowly dripped into my nose over a period of about 30 minutes. I will then lie on my side for two hours (during which time I can watch TV) after the gene is given in order to give the gene time to enter the cells inside my nose. Late in the afternoon of the same day, I will be asked to lie on my opposite side, and a similar solution which contains no CF gene will be administered in my other nostril. The reason for using both nostrils (one which receives the normal CF gene and one which does not) will be to help prove that any desirable effects which are observed are truly due to the normal CF gene, since they should occur only in the nostril which receives the normal gene.
4. Nasal potential difference measurements. The nasal potential difference measurement is performed in order to evaluate the movement of salts (such as sodium and chloride) across the tissue which lines the inside of the nose. Because individuals with CF have an abnormal nasal potential difference measurement, my potential difference measurement will be performed several times over the weeks before and after I am treated with the normal CF gene. The potential difference will be measured both in my nostril which receives the normal CF gene, and in the nostril which does not receive the gene, in order to verify that any changes which are observed are due to the presence of the normal CF gene in the cells lining the nose.

   The potential difference measurement is made by placing a needle under the skin of the forearm and then inserting a small piece of flexible tubing (again about the diameter of a kite string) between 2-6" into the nostril. The tubing will

very slowly drip a salt-water solution, which I might taste in the back of my throat during the measurement. For part of the measurement, the solution may also contain small amounts of drugs which help detect the presence of the normal CF gene. These drugs include Amiloride and Isoproterenol, two drugs which are already approved for use in humans and have been given to cystic fibrosis patients and other patients in the past. The measurement will be made approximately 15 times during the entire study. Each measurement requires about 10-15 minutes. The measurement can be made either while I am seated or lying down.

5. Nasal brushes and biopsies. Nasal brushes and biopsies will be performed in order to evaluate whether administration of lipid or DNA caused any damage to the tissues lining the nose. A small nasal brush will be used to scrape cells lining the inside of my nose. These cells will be studied for the presence of the normal CF gene, and to look for evidence of inflammation resulting from administration of the gene. This procedure can be slightly painful, with pain lasting from one to several hours. Numbing medication (Lidocaine) or over-the-counter pain medications will be used to control the pain. In order to further study whether administration of the normal CF gene causes inflammation or other damage, and to help show that the normal CF gene is being utilized by the cells lining the nose, two nasal biopsies will be performed on one occasion during the study. A local anesthetic (Lidocaine) will be given to numb the area prior to taking the biopsies. The nasal biopsy procedure can be associated with some bleeding, discharge, and local pain. This biopsy will be performed by an ear, nose, and throat physician.

6. Nasal Endoscopy
Prior to administration of the normal CF gene or other studies of the tissue inside the nose, a small rubber tube (an endoscope) will be placed about 4" inside the nose so that the tissues can be evaluated more easily. This procedure usually takes less than 10 minutes, and can be associated with minor discomfort. Local anesthesia or other pain medicine will be given whenever possible to control the discomfort.

With the exception of the nasal potential difference study, all of the tests and procedures described in this study are routinely performed at the UAB Hospitals. I understand that all of the test which I undergo will be only for the purpose of the CF Gene Transfer Study.

RISKS AND DISCOMFORTS

I am aware that there are several risks and discomforts associated with participation in this study. These include:

a) Pain, bruising and possible infection at the site of blood drawing, and at the site of insertion of the needle in the forearm as part of the nasal potential difference measurements.

b) Local pain and inflammation within the nose due to administration of the gene or

nasal potential difference measurement. Tearing and sneezing are also possible at the time the gene is given or otherwise studied. It is possible, but very unlikely, that inflammation, pain, change in sense of smell, or other damage to a small area of tissue within the nose could be permanent. However, it is much more likely that any symptom of pain or inflammation which develops would resemble a "cold" or "sinus infection" and would be temporary (for example, symptoms might include fever, runny nose or cough). The discomforts listed above will be minimized using numbing medications or other measures whenever possible.

c) Administration of the normal CF gene will require that I lie still on my side for approximately 60 minutes without moving my head, and lying on my side in bed for approximately four (4) hours.

d) The scrapings and biopsies of my nose can be painful, and may be associated with a small amount of bleeding. Healing of the nasal biopsy requires approximately two weeks and can be associated with pain, itching, runny nose, and/or minor bleeding. The risk of infection at the site of the biopsy is very small.

e) One chest x-ray and two sinus x-rays will be performed as part of the study. The chances of developing cancer or other complications from this amount of radiation is very small, although the actual risks in an individual CF patient who has received multiple x-rays in the past as part of usual care is not known.

SPECIAL RISKS AND DISCOMFORTS RELATED TO BEING PART OF A STUDY OF GENE ADMINISTRATION

Because CF gene transfer is experimental, the risks associated with this procedure are not known. Several risks are possible:

1. Administration of the normal CF gene using lipid could cause an allergic or inflammatory response which would make subsequent administration more difficult, or cause a significant allergic reaction to the lipid or the normal CF gene. This is very unlikely since lipid administration of the type planned here does not generally cause pronounced allergic or inflammatory reactions (for example, it has not caused substantial inflammation in studies in laboratory animals). In addition, the lungs of CF patients are already exposed to considerable amounts of other genes (in the form of DNA already present in the airways) so that administration of an additional gene is unlikely to provoke a significantly enhanced allergic response. However, the allergic reaction which might be caused in a given patient after gene administration is not known.
2. It is possible that the normal CF gene could enter cells other than those within the nose at the time of administration. For example, some blood cells could carry the CF gene to other parts of the body. In all gene transfer studies, a concern is that a new gene might enter a patient's sperm or egg. However, multiple barriers within the nose should make it very unlikely that significant amounts of lipid or DNA would be delivered to other areas of the body.

3. Because I do not have a normal CF gene, it is possible that if cells within my nose begin to use a normal CF gene, my body will recognize the CF protein which results as new and foreign, and attempt to destroy cells making the protein. I understand that animal studies have suggested that too much normal CF protein does not appear to cause any serious side effects, and although the risk is likely to be very small the effect in humans is unknown.
4. Other issues:
   a) I am aware that participation in Phase I studies such as this one sometimes may make it more difficult to gain entry into future experimental trials, although this is not intended by the physicians responsible for the study. I am aware that participation in this study could exclude me from later gene therapy or other experimental trials, until gene transfer has become a proven form of beneficial, and commercially available, therapy.
   b) Throughout the study I will continue to be monitored for complications which are normally associated with CF. Should any of these complications develop I will be given appropriate therapy. If the complications do not appear to be related to the gene transfer protocol, the usual means of payment (for example, insurance) should be arranged. UAB and The Children's Hospital of Alabama have made no provision for monetary compensation in the event of physical injury resulting from the research and in the event of such injury, medical treatment is provided, but is not provided free of charge.
   c) I have been informed that my participation in this study requires the practice of birth control because of the very small risk of the CF gene reaching the sperm or egg cell. If I conceive an infant unexpectedly during this study I will notify the medical researchers immediately.
   d) Despite the best efforts of the medical research team to maintain my confidentiality as part of this study, the public interest in gene therapy makes it possible that the news media or others could attempt to discover my identity. Although everything possible will be done to protect my privacy, if my identity is discovered through some other means I could be asked for interviews and it is possible that my privacy might be reduced in other ways due to my participation in this study.
   e) Because gene administration is a new form of therapy, close follow up with the medical researchers in this study will be required. Even after the five-month study is completed, I will continue to be followed for my cystic fibrosis, and will have a blood test and chest x-ray performed as part of my regular care approximately one year following gene transfer. I also understand that although the medical research team does not expect any substantial harmful effects to result from this study, the effects of gene transfer administration which I experience may be very important in terms of helping other patients with CF and possibly other diseases as well. This means that in the case of my death (whether this is due to a CF-related problem or to some other cause, such as an accident which has nothing to do with CF) an autopsy will be requested so that the effects of

gene transfer can be thoroughly studied.

BENEFITS

The primary benefit of my participation in this study will be an improved understanding of a possible new therapy for treating CF. I understand that the medical research team does not expect that administration of the normal CF gene to the inside of the nose will benefit me directly, but that this study could be an important first step towards new therapies in which the normal CF gene could be administered to the lungs of CF patients. Such future studies could be beneficial to myself and to other CF patients.

ALTERNATIVES

Standard cystic fibrosis therapy (chest physiotherapy, nutritional measures, antibiotics) as well as experimental therapies (rh DNase) are also available for the treatment of CF. If I decide not to participate in this study, it will not affect my care at the University of Alabama at Birmingham, and I will continue to receive the best available standard treatment for CF. I agree that by participation in this study, I will not enter another experimental study (for example, a rh DNase trial) at the same time. All standard CF therapy will remain available to me during my participation in this study. I understand that as a participant in this research study, if significant findings come forward during the course of the study which are relevant to my continued participation, I will be informed of these findings.

CONFIDENTIALITY

Every effort will be made to insure that information collected within this study will be kept confidential. I understand that federal, state, or local law could result in the release of my records, and that the Food and Drug Administration and associated organizations may also review the results of this study. My name will not be used in any report or publication concerning this study.

COST OF THE RESEARCH STUDY

I understand that I will not be charged for my participation in this study. If, despite all of the best precautions, a complication occurs as a result of this study, the medical research team will help me obtain appropriate medical care. If a health problem which is not caused by the study occurs while I am participating, I understand that the usual methods of payment (for

example, health insurance) should be sought.

RIGHT TO WITHDRAW

I may withdraw from this study at any time. I understand that I am participating on a voluntary basis and if I withdraw from this study this will in no way jeopardize my future care at the University of Alabama at Birmingham Hospital System and will not be associated with any loss of benefits. I also understand that Dr. Eric Sorscher or Dr. James Logan have the right to stop my participation in this study at any time. This could occur, for example, if I developed an unexpected problem or failed to follow directions.

AVAILABILITY OF INFORMATION

If I have any questions regarding this study, I understand that Dr. Eric Sorscher or Dr. James Logan will be available to answer my questions. Dr. Sorscher or Dr. Logan can be reached at (205) 934-4715. Dr. Sorscher's paging number is (205) 934-3411 (ask for beeper # 1135). Dr. Logan's paging number is (205) 939-9100 (ask for Dr. Logan).

WITNESS AND SIGNATURE

I have read and understand all of the information above, and have been given the opportunity to ask questions. I voluntarily agree to participate in this study. I understand that I will receive a copy of the Consent Form after it is signed.

_____

PRINTED Name of Patient

_____ _____

Patient Signature Date

_____ _____

Signature of Person Obtaining Consent Date

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Sorscher, Eric Jay	Assistant Professor

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Harvard Medical School, Boston, MA	M.D.	1984	
Yale University, New Haven, CT	B.S.	1979	Biology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1982-83 Candidate took one year leave of absence from Medical School to pursue research in cystic fibrosis. Research involved ion transport investigations with measurements of respiratory mucosal potential difference and also clinical trials with experimental drugs at Children's Hospital, Boston, MA.

1984-87 Resident; Department of Internal Medicine, Massachusetts General Hospital

1987-88 Postdoctoral Fellow; Department of Physiology and Biophysics, University of Alabama at Birmingham

1988-89 Postdoctoral Clinical Fellow; Department of Medicine, Division of Hematology and Oncology, Massachusetts General Hospital

1989-present Assistant Professor, Department of Medicine, (Primary appointment); Assistant Professor, Department of Physiology & Biophysics, (Secondary appointment); Associate Scientist, Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama at Birmingham

PROFESSIONAL AND SCIENTIFIC SOCIETIES

American Board of Internal Medicine
American Society of Hematology

HONORS

1979 Phi Beta Kappa
Summa cum laude with honors in Biology, Yale University

1982-83 Research Fellowship Award: Harvard Medical School
Research Scholar Award: National Cystic Fibrosis Foundation

1987-88 Postdoctoral Fellowship Award: National Cystic Fibrosis Foundation

1989-present Lucille P. Markey Scholar in Biomedical Sciences

RECENT SYMPOSIA/INVITED PRESENTATIONS

International Cystic Fibrosis Conference, Williamsburg, VA June, 1993
Science Innovation/American Association for the Advancement of Science Boston, MA August, 1993
Young Leaders Conference, United States Senatorial Nomination to Serve as Alabama Delegate, Basel, Switzerland September, 1993

PUBLICATIONS (Articles)

Sorscher E.J. and Brewlow J.L.. Cystic fibrosis: A disorder of calcium stimulated secretion and transepithelial sodium transport? Lancet 1:368-370, 1982.

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Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

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9312-067 Welsh, Michael J.; Howard Hughes Medical Institute, Iowa City, Iowa; *Adenovirus-Mediated Gene Transfer of CFTR to the Nasal Epithelium and Maxillary Sinus of Patients with Cystic Fibrosis.*

Date of RAC Approval: 12/3/1993
Date of NIH Approval: 2/10/1994

1. SCIENTIFIC ABSTRACT OF THE PROTOCOL

This protocol is an extension of an earlier one in which a defective adenovirus vector (Ad2/CFTR-1) was applied to the nasal epithelium of three CF patients. In all three cases changes in electrolyte transport were detected following treatment with vector. No adverse effects that could be attributed to the adenovirus vector were detected. This protocol seeks to address the next key questions regarding the use of adenovirus vectors, first is it safe to administer virus multiple times and is it possible to obtain evidence of clinical as well as biochemical evidence of efficacy?

This protocol will use a second generation adenovirus vector named Ad2-ORF6/PGK-CFTR. This virus lacks E1 and in its place contains a modified transcription unit with the phosphoglycerate kinase (PGK) promoter and a poly A addition site flanking the CFTR cDNA. The PGK promoter is of only moderate strength but is long lasting and not subject to shut off. The E4 region of the vector has also been modified in that the whole coding sequence has been removed and replaced by ORF6, the only E4 gene essential for growth of Ad2 in tissue culture. This has the effect of generating a genome of 101% the size of wild type Ad2 and renders the vector more easy to grow in culture than Ad2-ORF6/PGK-CFTR.

The protocol involves production of Ad2-ORF6/PGK-CFTR-1 virus in 293 cells that have been extensively tested for adventitious agents, using a viral seed stock that has been similarly tested. Following purification and further testing, the Ad2/CFTR-1 stock will be applied directly to the nasal epithelium or to the maxillary sinus.

The protocol has two parts. In the first, Ad2-ORF6/PGK-CFTR will be applied in increasing doses (2×10^7, 2×10^8, 2×10^9, and 2×10^{10} (twice)) to one nostril at approximately monthly intervals. Saline will be applied to the other nostril. In the second part of the protocol, virus will be applied to one submaxillary sinus up to seven times (5×10^8 (1x), 2.5×10^9 (up to 6x)). The contralateral sinus will act as a control. Five to ten patients will be recruited to each protocol, and patients will be carefully assessed for evidence of inflammation and virus replication following administration. Measurements of transepithelial voltage will be taken on the patients undergoing the nasal protocol and antibody titre to adenovirus will be studied. At the end of the first part of the protocol, biopsies of both nostrils will be taken. In the second part of the protocol, the sinus will be lavaged and treated with antibiotics and following the administrations of virus, evidence of reduced edema, bacterial infection and/or mucus production will be taken as indicators of clinical efficacy. Sinus biopsies will be taken at the end of the second part of the protocol.

Participants will be patients with CF who are at least 18 years old and have mild to moderate disease. We prefer patients homozygous for the common ΔF508 mutation. We will require patients to be seropositive for adenovirus antibody and to have no evidence of respiratory viral infections for the prior two weeks. Following treatment, patients will remain isolated in the hospital for 24 hours.

The successful outcome of this protocol will add further to our knowledge of the safety and efficacy of adenovirus vectors and will be invaluable in the design of subsequent protocols targeted to the respiratory airways and of future generations of adenovirus vector.

2. DESCRIPTION OF THE PROPOSED EXPERIMENT IN NON TECHNICAL LANGUAGE

Cystic fibrosis (CF) is a common inherited disease among the white population of Europe and North America. It is characterized by chronic infection and obstruction of the airways leading to the lungs. Patients usually die from the disease by their mid-to-late twenties. The recent discovery and characterization of the inherited material - the gene - that causes the disease has lead to a rapid increase in our knowledge of the basic defect responsible for the disease state, and has raised the possibility of treating CF by giving to the patient a good copy of the CF associated gene. This is a new approach to the treatment of inherited diseases, called gene therapy.

Eventually gene therapy for cystic fibrosis might be achieved by treating the airways of CF patients with a "tailor-made" virus that has been altered to make it less infectious and to include the CF gene. One possible virus to do this is called adenovirus. It is normally associated with minor respiratory infections, such as colds. Although adenoviruses have been used safely in the past as vaccines, we need to establish the feasibility and safety of using such an approach.

The authors of this proposal have already treated three CF patients with very small amounts of CF-adenovirus and shown it is possible to correct the basic defect associated with the disease. Virus was applied to a small area within the nose and a small electrode used to measure the tiny voltage that normally exists across the lining of the nose. In patients with CF, this voltage is abnormal because of a defect in the CF gene. After administration of virus, this voltage was restored to normal for the few days tested.

We now hope to extend these studies to ascertain whether it is safe to administer larger amounts of virus, whether it is safe to administer it more than once, and whether we can detect evidence of clinical improvement. To do this, virus will be applied to the nose or to the sinus. By using these organs, we can obtain the data we wish to measure without using large amounts of virus or using invasive clinical protocols. In this way, we hope to maximize patient safety, while at the same time determining whether adenovirus is likely to be safe and to be efficacious.

APPLICATION TO
NATIONAL INSTITUTES OF HEALTH
RECOMBINANT DNA ADVISORY COMMITTEE

TITLE

ADENOVIRUS-MEDIATED TRANSFER OF CFTR TO THE NASAL EPITHELIUM AND MAXILLARY SINUS OF PATIENTS WITH CYSTIC FIBROSIS

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- 7.5 Manuscript: *Development and Analysis of Recombinant Adenoviruses for Gene Therapy of Cystic Fibrosis,* Rich, et al. (1993).
- 7.6 Manuscript: *Safety and Efficacy of Repetitive Adenovirus-Mediated Transfer of CFTR cDNA to Airway Epithelia of Primates and Cotton Rats,* Zabner, et al. (1993).
- 7.7 Manuscript: *Efficiency of Adenovirus-Mediated Transfer of CFTR at Correcting cAMP-Stimulated Fluid Secretion and Chloride Transport in Cystic Fibrosis Airway Epithelia In Vitro,* Zabner, et al. (submitted for publication).
- 7.8 Manuscript: *Adenovirus-Mediated Gene Transfer Transiently Corrects the Chloride Transport Defect in Nasal Epithelia of Patients With Cystic Fibrosis,* Zabner, et al. (submitted for publication)

8.0 Curricula Vitae

ADENOVIRUS-MEDIATED GENE TRANSFER FOR CYSTIC FIBROSIS:
Part A. Safety of Dose and Repeat Administration in the Nasal Epithelium
Part B. Clinical Efficacy in the Maxillary Sinus

Investigator: Michael J. Welsh, M.D.
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INTRODUCTION

Cystic fibrosis (CF) is a common lethal autosomal recessive disease of Caucasians (1-3) caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (4-6). CFTR is a chloride channel which is regulated by phosphorylation and by intracellular nucleotides (7). Mutations in the CFTR gene cause a loss of function of the CFTR chloride channel and thus contribute to the hallmark of the disease: defective electrolyte transport by affected epithelia (1,8). Although there are a variety of clinical manifestations, lung disease is the major cause of morbidity, and despite current standard therapy the median survival rate is only 26 years. Recently obtained knowledge of the gene that encodes CFTR, an understanding of the function and biochemistry of the protein product, and insight into the molecular basis of the disease (3,7,9,10), suggest that gene transfer could represent an important advance in treatment.

The feasibility of gene transfer to lung cells was initially demonstrated by our finding that expression of the cDNA for wild-type CFTR corrected the chloride channel defect in cultured CF airway epithelia (11). In collaboration with Dr. Alan E. Smith and his colleagues at Genzyme Corp., we constructed and tested recombinant adenovirus vectors to deliver CFTR cDNA to airway epithelial cells in cell culture models and in animals; papers describing the results of those studies are included as Appendices 7.5, 7.6, and 7.7.

Based upon the results of those studies and additional work, we received approval from the University of Iowa IRB and IBC, the NIH RAC, and the FDA for a single dose nasal application of an adenovirus-based vector encoding CFTR to three patients with CF. We have now completed that study and a manuscript describing the results is included as Appendix 7.8. We found that administration of an adenovirus vector corrected the chloride transport defect that is the hallmark of CF-affected epithelia. We have also received IRB and IBC approval for the study of three additional patients under an addendum to that protocol; approval of the NIH RAC and the FDA is pending.

We are now requesting approval for a study that is designed to address two of the most pressing issues regarding the use of adenovirus vectors for the treatment of CF airway disease.

In Part A, we address the safety of dose and repeat administration to the nasal epithelium. There are two main issues: a) Is repetitive administration safe? Because adenovirus rarely integrates, gene therapy for CF will require repeated vector administration. b) Is administration of increased doses safe? In our initial study (Appendix 7.8), we used low doses of vector, but transfer of CFTR cDNA to the intrapulmonary epithelium will require higher doses.

In Part B, we ask whether delivery of the an adenoviral vector encoding CFTR is of clinical efficacy. We will deliver AD2-ORF6/PGK-CFTR to the sinus mucosa of five to ten CF patients to learn if Ad2-ORF6/PGK-CFTR can treat CF clinical disease in the maxillary sinus. We have chosen the maxillary sinuses because the morphology, function, and clinical abnormalities are similar to those found in the intrapulmonary airways. The study will also allow us to determine if repeat administration of an adenovirus vector is safe. The proposal is designed to answer these questions in a way that minimizes risk to the patients, yet provides guidance for the future direction of gene therapy to treat CF.

BACKGROUND AND RATIONALE

In our previously approved RAC and IRB protocol (Cystic Fibrosis Gene Transfer: *In Vivo* Safety and Efficacy in Nasal Epithelium) we provided a discussion of CF and CF lung disease as background and provided substantial references as documentation. We will not repeat that here. The major points are:

a) Cystic fibrosis is a common genetic disease (1).
b) Although there are a variety of clinical manifestations, severe chronic lung disease is the major cause of morbidity and mortality.
c) Although improvements have been made, no treatment is directed at the underlying defect. As a result CF remains a life-threatening and often lethal disease.

CONSIDERATIONS FOR GENE THERAPY OF CF

In our previously approved IRB protocol (Cystic Fibrosis Gene Transfer: *In Vivo* Safety and Efficacy in Nasal Epithelium), we provided a substantial discussion of issues pertinent to gene therapy of CF and supporting references. We provide only a very brief summary here. A more extensive discussion is contained in Appendices 7.5 - 7.8 and in reference (1).

Target Tissue for Gene Transfer.
Because 95% of CF patients die of lung disease, the lung will be the main target for gene therapy. Within the lung, the target tissue for gene transfer that is most likely be of benefit to a patient with CF is the airway epithelium. Loss of function of CFTR within the airway epithelium is thought to contribute directly to the pathogenesis of the disease.

It is unlikely that any gene therapy protocol will correct 100% of the cells that normally express CFTR. Our data in cultured cell models and that of other investigators suggests that correction of a fraction of the cells may be sufficient to at least partially correct the chloride transport defect (Appendix 7.7). Although the safety of overexpressing CFTR is not known with certainty, current data suggests that there may be a reasonably wide therapeutic index.

Adenovirus Vectors for Gene Transfer to Patients with CF.
Adenovirus has a number of advantages as a vector for gene transfer to the airway epithelium; these are enumerated in Appendices 7.5 - 7.8 and our previous RAC application. Adenoviruses also have an impressive safety profile in terms of the toxicity of wild-type infections, their use in vaccines, their lack of oncogenicity in humans, and our initial trials in humans. Adenovirus also has some potential disadvantages, the one of most concern relates to the possibility that administration of adenovirus may induce an inflammatory or immune response. An additional concern is the possibility of virus replication.

Use of Nasal Epithelium to Assess Safety and Biochemical Efficacy.
The use of nasal epithelium confers several advantages for our studies; we discussed these in detail in our previous RAC protocol and in Appendix 7.8. They include: a) The epithelium has the same morphology, and electrolyte transport as intrapulmonary airway epithelia. b) Upper and lower airway epithelia have the same defect in electrolyte transport in CF. c) The epithelium is accessible for vector administration, inspection, and sampling, thereby allowing relatively noninvasive evaluation. d) The risk to participants is minimized if an adverse reaction should occur. e) The effect of expressing CFTR Cl^- channels in the apical membrane of the epithelium is readily assessed by measuring the *in vivo* transepithelial voltage (12) and its response to cAMP agonists. f) Any adverse effects of vector administration can be readily assessed by inspection, by sampling cells, cytokines, and inflammatory mediators, by assessing surface immunoglobulins, and by a full-thickness biopsy of mucosa.

Evaluation of Clinical Efficacy of Gene Transfer in CF.
A number of potential problems can be anticipated in the evaluation of clinical efficacy of gene transfer in the lung. As a result, it may be difficult to determine whether clinical benefits outweigh any adverse effects associated with vector administration. Because CF is a chronic disease, tests of severity made over a short period of time may not be sensitive or may be subject to error. If one chooses to study patients with severe disease (FEV1 of 20-30% predicted), then there will be a large amount of irreversible bronchiectasis and other pathology that would not be affected by gene transfer. Moreover, the two year

mortality rate for those patients is 50% (13). In contrast, those patients with mild lung disease (FEV1 > 61%) have mortality rates of less than 5% in two years. Thus, assessment of mortality is impractical, as an endpoint in evaluation of therapy.

Pulmonary function tests are frequently helpful as an endpoint but they are not without problems. They are subject to fluctuations by factors other than those being investigated. Perhaps evaluation by high-resolution chest CT, assessment of mucociliary clearance, and cardiopulmonary exercise testing may be more sensitive and specific.

Use of Maxillary Sinus Epithelium to Assess Clinical Efficacy.
An alternative to evaluate clinical efficacy is to use the maxillary sinuses. The upper airways of the nose and sinuses are almost universally involved by CF. In fact some have suggested that the absence of pansinusitis rules out a diagnosis of CF. Because we propose below to use the upper airways and sinuses in our evaluation of gene transfer for patients with CF and because knowledge of the upper airways in CF may be less widespread than knowledge of the intrapulmonary airways, we briefly review this subject with particular emphasis on the sinuses. Wine et al. (14) were the first to suggest use of the sinuses to evaluate therapeutic interventions in CF.

Anatomy. The paranasal sinuses include the maxillary, frontal, ethmoid, and sphenoidal sinuses. All are involved by CF. The frontal sinuses rarely develop in CF patients, probably because of early onset of sinusitis which prevents pneumatization (15). In this proposal we focus on the maxillary sinuses which are uniformly involved in CF. The maxillary sinuses are connected to the nasal cavity through the maxillary sinus ostium. Nasal polyps have been reported in 10-32% of patients (16).

Histology. The maxillary sinuses are lined by respiratory epithelium with ciliated cells, nonciliated cells, basal cells, and goblet cells (17). The epithelium in CF sinus disease is very similar to that observed in CF intrapulmonary disease. The histology of CF polyps are also similar to polyps associated with allergy in that there is mucus gland hyperplasia and mucus cysts. It has been suggested that CF polyps are distinct from allergic polyps because they have a relatively normal basement membrane and few eosinophils. However, such changes are also found in non-CF polyps and in blinded studies it is difficult to distinguish between polyps from the two diseases (18,19).

Submucosal glands in upper airway epithelium. Careful quantitative evaluation of submucosal glands in upper and lower human airways indicates that glands in the sinus, nasal, tracheal and bronchial mucosa have similar histologic and morphology properties. The density of glands in the maxillary sinus of adult humans is 0.2 glands/mm^2. For comparison, the density of glands in the adult trachea is 0.8-1.0 glands/mm^2, but the density decreases as one moves distally from the trachea into the bronchi (20). Thus, the submucosal glands in the large and small airways are similar in morphology and number to those in the maxillary sinuses.

Clinical presentation. Despite almost universal involvement of the sinuses in CF, the clinical presentation is often subtle. The majority of CF patients do not have symptoms as a result of sinus disease. When symptoms do occur, the most common are headache, nasal obstruction, purulent rhinorrhea, mouth breathing, snoring, postnasal drainage with cough, and constant throat clearing (16,21-23). Occasionally acute sinusitis may develop.

Physiology. Most importantly for our studies, CF causes similar abnormalities of electrolyte transport in both upper and lower airway epithelia. Both manifest a lack of CFTR chloride channel function. In fact, the first evidence to suggest that CF airway epithelia had defective electrolyte transport was the study of upper airway epithelia (12). Chloride transport function can be assessed by measuring the voltage across the airway epithelium. This assay provides an easy and reliable measure of Cl^- transport by the epithelium (12,24,25). The pathogenesis and pathophysiology of sinus disease and nasal polyposis in CF is thought to be identical to that of the pulmonary disease: altered respiratory tract fluid leading to impaired mucociliary clearance.

Bacteriology. Studies in laboratory animals and a small number of humans have shown that the maxillary sinuses are normally sterile. But in CF, the maxillary sinuses are infected with the same organisms that cause lung disease: the most common is *Pseudomonas aeruginosa* (14,16,26). As with

CF lung disease, antipseudomonal antibiotics alone are not sufficient to eradicate sinus infection in patients with CF.

Radiology. Coronal sinus computed tomography (CT scans) are the most sensitive way to diagnose and evaluate sinus disease. In the vast majority of cases maxillary sinuses are opacified as a result of mucosal thickening and thick tenacious secretions which occupy the lumen. The periosteum can also be involved (14,27).

Treatment. In most asymptomatic cases, treatment of sinus disease is not required. When treatment is required, antibiotics are usually administered. But in most patients antibiotic treatment alone is not successful, and surgical drainage is performed. While 50-70% of non CF patients with chronic sinusitis respond well to endoscopic sinus surgery, nearly all tend to relapse after initial success. For example, in a three year prospective study, seven CF patients underwent endoscopic polypectomy and enlargement of the maxillary antra (28). There was good relief initially, but a coronal CT scan performed three years after surgery revealed severe pansinusitis and opacification of maxillary sinuses.

Relationship between sinus and lung disease. Chronic colonization of the paranasal sinuses with *Pseudomonas* and other organisms may contribute to CF lung disease. Drainage of organisms from the sinuses into the dependent lung is a source of colonization and inflammation. A similar situation occurs with sinus disease in non CF subjects who develop secondary cough, bronchial reactivity, and asthma (29). CF patients who have undergone heart-lung transplantation can develop recurrent *Pseudomonas* bronchitis in the transplanted lung. But treatment with maxillary sinus antrostomy and repeated sinus lavage with antibiotics produced significant improvement and prevented the lung disease (30). In addition, adult CF patients who underwent bilateral Caldwell-Luc procedures to remove the maxillary sinus mucosa showed significant improvement in both local and respiratory symptoms and had a decreased frequency of hospital admission for pulmonary exacerbations (31). These observations suggest that sinus disease may contribute to pulmonary exacerbation in patients with CF.

Safety advantages of using upper airway epithelium. Use of the sinus epithelium will provide several safety advantages. a) It will allow us to use a relatively small total amount of virus because we can apply it to a limited area. As a result, we will be able to obtain a great deal of useful information, but at the same time, use of a small amount of virus will minimize the risk. b) Upper airways have the advantage that the epithelium is more readily accessible than that in the lower airway for the frequent studies required to test safety and efficacy. Because it is more accessible, there will be less patient discomfort and risk. c) If significant inflammation or cytotoxicity should develop as a result of administration of the recombinant virus, the consequences to the patient will be much less severe if they occur in the upper airways than if they occur more generally in the lung.

Advantages for assessing efficacy. Use of the maxillary sinus epithelium provides several advantages for assessing the clinical efficacy of gene transfer. As we indicate above, because CF is a chronic disease, testing clinical efficacy of gene transfer by assessing lung disease may be difficult, yet knowledge that the clinical defect can be corrected is critical to future efforts at gene transfer for CF lung disease. Testing clinical efficacy in the maxillary sinuses has the advantage that the tests to assess function (sinus CT and cell counts and inflammatory mediators in sinus washes) may be more sensitive and yet less invasive than those required to test clinical efficacy in the lung.

Resolution of chronic sinusitis. Resolution of chronic sinusitis involves not only the elimination of chronic bacterial growth but also the re-establishment of mucociliary transport and the resolution of edema. In non CF patients this usually takes 4-6 weeks. We are not certain how long it may take to observe improvement of CF sinus disease after transfer of CFTR cDNA, but we expect that if clearing is to occur, it may require from 6 weeks to 6 months. Because the sinuses are markedly abnormal by coronal CT scans, improvement over baseline may be obvious to independent radiologists. Quantitative cultures of sinus lavage for *Pseudomonas aeruginosa* and measurement of inflammatory mediators will also be valuable endpoints.

Summary. In summary, we believe that a trial of clinical efficacy using the maxillary sinuses has significant advantages: a) The maxillary sinuses provides an excellent model of lower airway epithelium in CF. They have similar histology and submucosal glands, and the physiology and pathophysiology are

similar. b) Assessment of CF clinical disease is easier and entails less risk than equivalent assessment of lung disease. c) Because there is no physical connection between the two maxillary sinuses, we can use one sinus as a control. d) The dose of virus is relatively small because of the limited area of application. e) The risk to participants is minimized if an adverse reaction should occur in the sinuses as compared to the risk with lung administration.

DESIGN OF Ad2-ORF6/PGK-CFTR.

The vector to be utilized in the present protocol was derived from Ad2 DNA and CFTR cDNA. It is named Ad2-ORF6/PGK-CFTR. The DNA construct comprises a full length copy of the Ad2 genome of approximately 37.5 kb, from which the early region 1 genes (present at the 5' end of the viral DNA) have been deleted and replaced by an expression cassette encoding CFTR. The expression cassette includes the promoter for phosphoglycerate kinase (PGK) and a poly A addition site from the bovine growth hormone gene. In addition, the E4 region of Ad2 has been deleted and in its place the open reading frame 6 of the Ad2 E4 region has been inserted. Appendix 7.1 describes in detail the design and construction of the Ad2-ORF6/PGK-CFTR vector.

STUDIES OF Ad2-ORF6/PGK-CFTR.

Studies of Ad2/CFTR-1, Ad2/ßGal-1, and Ad2/CMV-ßGal.

Many of our studies of the related vectors - Ad2/CFTR-1, Ad2/ßGal-1, and Ad2/CMV-ßGal - are described in Appendices 7.5-7.7 and our previous RAC proposal. The experiments include studies of the life cycle, safety, and efficacy of the vectors *in vitro*, as well studies in cotton rats, guinea pigs, and Rhesus monkeys. Appendix 7.8 describes the results of our first study using Ad2/CFTR-1 in humans. These studies are encouraging in terms of the ability to express and in terms of safety.

Studies of Ad2-ORF6/PGK-CFTR *In Vitro*.

We have tested the ability of Ad2-ORF6/PGK-CFTR to express CFTR in several cell lines, including human HeLa cells, human 293 cells, and primary cultures of normal and CF human airway epithelia. When these cells were grown on culture dishes, the vector was able to transfer CFTR cDNA and express CFTR as assessed by immunoprecipitation and by functional assays of halide efflux. We have also shown that Ad2-ORF6/PGK-CFTR can correct the cAMP-stimulated chloride secretion defect in primary cultures of CF airway epithelial cells grown on permeable filter supports at the air-liquid interface (32-35). Such cultures closely resemble the native epithelium, both morphologically and functionally. Importantly, the results of our first study in humans with CF (Appendix 7.8), indicate that our data obtained with primary cultures of human airway epithelia grown on permeable filter supports predict the results in the airway epithelium of patients.

In studies of the life cycle of Ad2-ORF6/PGK-CFTR, we found that viral DNA remains episomal. There was no evidence of viral DNA synthesis and no evidence of DNA instability. We have been unable to detect viral replication except in the permissive 293 cells. The functions of E1 could, however, be complemented by superinfection with a wild-type virus. To examine viral DNA synthesis in doubly infected 293 cells we took samples at different times following infection with wild-type and the Ad2E4$^-$ORF6$^+$ virus and examined adenovirus DNA following restriction enzyme digestion. We found that wild-type DNA can be detected by the appearance of characteristic DNA fragments and that these fragments rapidly overgrow the Ad2E4$^-$ORF6$^+$ backbone virus. This result implies that although the Ad2E4$^-$ORF6$^+$ virus is viable in 293 cells, its growth is disabled relative to wild-type. This is perhaps not surprising since it lacks E4ORF6/7 and ORF3. These results argue that Ad2-ORF6/PGK-CFTR may be disabled relative to wild-type in the event of an inadvertent subsequent infection of a treated patient with wild-type virus.

We have examined the preparations of Ad2-ORF6/PGK-CFTR by the HeLa cell assay and by the PCR reaction described above. We have not detected any wild-type recombinant virus by these assays. Thus, at the sensitivity levels of these assays we have not detected any recombination events. We are currently refining these assays to enable us to demonstrate the absence of wild-type adenovirus in the highest doses proposed here. Results of *in vitro* studies are described in detail in Appendix 7.2-7.3.

Studies of Ad2-ORF6/PGK-CFTR *In Vivo*.
We have used cotton rats (*Sigmodin hispidus*) for several studies because they have been shown to be permissive for viral replication after intranasal inoculation of wild-type adenovirus types 1, 2, 5, and 6 (36,37). Treated cotton rats also develop pneumonia and thus can mimic human pathology induced by adenovirus. In Appendix 7.4, we describe studies in which we have investigated the efficacy of adenovirus vectors and the safety of Ad2-ORF6/PGK-CFTR during repeated administration. Although we observed some variability in expression, we observed no difference in expression of recombinant protein despite 4 repeated administrations of an adenoviral vector. More importantly, although we observed occasional patchy inflammation as indicated by a macrophage infiltrate, it did not correlate with treatment, i.e.., it was the same in treated and control animals.

We studied Rhesus monkeys (*Macaca mulatta*) because the physiology and anatomy of the airway epithelium closely resembles that of humans (38). For our proposed studies in humans, we plan to use the upper airway epithelium. Therefore, we tested the safety and efficacy of repeat administration of adenovirus vectors in the nasal epithelium. We applied Ad2/CFTR-1 on three occasions (results reported in Appendix 7.6). Then we applied Ad2-ORF6/PGK-CFTR to the nasal epithelium on two separate occasions for a total of 5 administrations. Although all three monkeys had positive antibody titers to adenovirus, none of the monkeys developed any clinical signs of viral infection or inflammation, either systemically or in the nasal epithelium. There was no viral replication and biopsies and brushings of treated epithelium showed no evidence of inflammation. Repeated treatment did not prevent expression of CFTR. Results of *in vivo* studies are described in detail in Appendix 7.4

SPECIFIC AIMS

The foregoing considerations indicate that gene therapy would be a major advance in the treatment of CF. They also suggest that adenovirus may be a good vector system for transfer of CFTR cDNA to correct the CF pulmonary defect. However, use of adenovirus vectors as a treatment for CF lung disease will require repeat administration and administration at higher doses than we have previously used in our initial study in humans. Moreover, the use of and further development of adenoviral vectors will require some demonstration of clinical efficacy in CF airway epithelia involved by the disease. Therefore, in this application we propose to address these issues.

Part A. Safety of Dose and Repeat Administration in the Nasal Epithelium.
1. Is repeat administration of an adenoviral vector safe? We will examine the effect of repeat administration of Ad2-ORF6/PGK-CFTR to the nasal epithelium to determine if it causes an inflammatory or immune response. We will also determine if the efficacy of Ad2-ORF6/PGK-CFTR in correcting the CF chloride transport defect decreases with repeated administration.
2. What is the effect of vector dose on safety and efficacy? We will examine the effect of progressively increasing doses of Ad2-ORF6/PGK-CFTR on safety and biochemical efficacy.

Part B. Clinical Efficacy in the Maxillary Sinus.
1. Does adenovirus-directed CFTR expression have clinical efficacy? We will test the clinical efficacy of Ad2-ORF6/PGK-CFTR in correcting the airway epithelial disease observed in the maxillary sinus. We will examine the effect of repeat administration, use one maxillary sinus as a control, and assess clinical efficacy using computed tomography scans as well as cellular and biochemical measures of efficacy. The data on clinical efficacy will also assess the balance between efficacy in correcting the biochemical defect and any potential adverse effects associated with vector administration.
2. Is repeat administration of an adenoviral vector safe? We will examine the effect of repeat administration of Ad2-ORF6/PGK-CFTR to determine if it causes an inflammatory or immune response.

STUDY PLAN, PART A

Recombinant Adenovirus Directing Expression of Human CFTR.
The recombinant adenoviral vector/CFTR gene construct, Ad2-ORF6/PGK-CFTR, will be made at Genzyme Corporation, Framingham, MA. It will be tested at Genzyme Corporation and at outside contractors, including Microbiological Associates, Bethesda, MD.

Study Design.
This is a non-randomized, non-blinded administration of recombinant Ad2-ORF6/PGK-CFTR to respiratory nasal epithelium. Although administration of vector will not be blinded, the analysis of many of the results will be blinded, using the saline-treated contralateral nasal mucosa as a control.

Number of Participants.
We intend to study five to ten patients. All patients will be identified in the first year. Patients will be referred to the study for consideration by their physicians.

Selection Criteria.
Inclusion Criteria
a) Patients with CF with mild to moderate severity of disease. Patients should score above 70 using the CF NIH scoring system (39).
b) Male or female patients, age greater than 18 years.
c) CF genotype: ΔF508 homozygous is preferred. If patients homozygous for ΔF508 are not available, we will treat patients with other defined genotypes known to be associated with CF.
d) Seropositive for antibody to adenovirus. Seropositivity is very common (greater than 70%) in the general population (40). Seropositivity is required to insure a rapid and adequate immunologic response to the virus and to minimize any potential for dissemination of the virus.
e) We will not exclude subjects who have participated in our first gene transfer protocol involving a one time, low dose administration of Ad2/CFTR-1.

Exclusion Criteria
a) Current instability of respiratory status.
b) Hypoxemia with P_aO_2 less than 70 mm Hg.
c) FEV_1 less than 50 % of predicted.
d) Weight for height (%) less than 75 % of predicted.
e) Pregnancy: all subjects must practice contraception for at least one month before the study and then during the course of the study. Women must have a negative test for pregnancy.
f) Patient has school age children.
g) Chronic severe nasal/sinus disease, either infectious or allergic in nature. Persistent purulent nasal discharge, obstructing nasal polyps, significantly inflamed nasal mucosa, or symptomatic sinusitis requiring repeated antibiotic therapy will exclude patients from the study.
h) Upper respiratory infection of patient or household member within two weeks prior to entry.
i) Chronic adenoviral shedding within three weeks of study as detected in blood, urine, and nasal swabs.
j) Patients with a known allergy to Xylocaine or Pontocaine or a bleeding diathesis.
k) Subjects may not participate in other research protocols during the proposed study.
l) Inability to comprehend nature of the study or to give informed consent.

Timing of Evaluations.
To insure clinical stability, participants will enter the study at least 3 weeks before administration of virus. They will then be evaluated at the times indicated on the attached flow sheet. The specific evaluations are listed below. It is possible that the first pretreatment evaluation could be longer than three weeks before Ad2-ORF6/PGK-CFTR administration. At the discretion of the investigators participants may also receive any of the evaluations between enrollment and administration of the recombinant virus if there is a significant change in clinical status.

Vector Application.
Patients will be admitted to the Clinical Research Center at the University of Iowa. After vector administration, they will be placed in isolation in a hospital room for one day. They will be discharged from the hospital the day after Ad2-ORF6/PGK-CFTR administration.

We will administer Ad2-ORF6/PGK-CFTR by directly applying it to the nasal epithelium over the course of 30 min. We will use the epithelium of the entire nasal cavity, which we estimate to be 20 to 50 cm^2. On the first administration, the nasal epithelium that will receive Ad2-ORF6/PGK-CFTR will be chosen at random. We will administer saline to the opposite nasal epithelium. After administration, we will remove any excess fluid. The same nasal cavities will be treated with Ad2-ORF6/PGK-CFTR or saline on all subsequent administrations.

Dose of Vector.
Each participant will receive the same doses of Ad2-ORF6/PGK-CFTR:

Administration	Total IU
1	$2x10^7$
2	$2x10^8$
3	$2x10^9$
4	$1x10^{10}$
5	$1x10^{10}$

Timing Of Repeat Administrations.
We estimate, based on our previous studies in non-human primates and in humans with Ad2/CFTR-1, that the duration over which an adenovirus vector may correct the CF Cl^- transport defect will be approximately 4 weeks, but could be substantially longer. The planned interval between vector administrations will be 4 weeks. However, the interval may be extended at the discretion of the investigators if the correction of the Cl^- transport defect persists beyond 4 weeks or if unavoidable problems should arise. For Part A of this study which is focused on safety, precise timing between administrations will not be critical to the evaluation or interpretation of the data. However, the shortest duration between treatments under any circumstances will be 4 weeks.

List Of Evaluation Procedures.
History and physical examination.
Purpose: To obtain evidence of patient discomfort, systemic responses, or inflammation.
Methods: The patient will be questioned about local or systemic manifestations of inflammation. Specific attention will be placed on symptoms of nasal congestion. Vital signs will be recorded. The area of application of the recombinant virus will be visually examined by endoscopy and compared to nontreated epithelium. In many cases the area of administration will be photographed to provide documentation. We will look for evidence of exudate, inflammation, edema, or erythema.

Blood/serum by venipuncture.
Purpose: To assess the systemic response to virus application and the antibody response to recombinant virus.
Methods: Venous blood will be collected by standard venipuncture technique. Analysis will include: complete blood count (including platelets and differential WBC), erythrocyte sedimentation rate, electrolytes (including Na, K, Cl, and HCO_3), and general chemistries (including total protein, albumin, Ca, PO_4, glucose, uric acid, alkaline phosphatase, total bilirubin, AST, LDH, BUN, Cr, and amylase). PT and PTT will be evaluated at the times indicated. Antibody titers to adenovirus will be determined by ELISA and by determination of neutralizing antibodies.

Viral cultures of nasal and pharyngeal swabs, urine, and stools.
Purpose: To determine the presence of recombinant virus.
Methods: All samples will be cultured on virus-permissive 293 cells. Based on our studies in animals, we expect that live virus could be present in the initial swabs but then would disappear with time. Our initial studies in humans (Appendix 7.8) suggest that Ad2/CFTR-1 could no longer be detected by culture

one day after administration. If there is viral replication, we expect that after an initial decline, the titer of live virus in the nasal swab would increase. We will determine whether wild-type or recombinant virus is produced by culture on HeLa cells, by restriction enzyme analysis, and by sequencing.

Swab of nasal mucosa for evaluation of an inflammatory response.
Purpose: To determine the presence of an inflammatory response.
Methods: The nasal mucosa from each nostril will be swabbed with a cotton-tipped applicator. The cells will be dissociated into 2 ml of phosphate buffered saline. The cell suspension will be kept on ice until further use. Evidence of an inflammatory response will be assessed by cytological examination of cytospin preparations of the cells using Wright stain. The cell differential count will be determined for each specimen. Cell morphology and cytopathic effects will be evaluated using the PAP stain.

Transepithelial electrical potential difference across the nasal epithelium.
Purpose: To assess the electrophysiological consequences of treatment. Patients with CF have an abnormally increased transepithelial electrical potential difference across the nasal epithelium (Vt) (12,24). In addition, the response of Vt to several agents that regulate transport is abnormal. The abnormalities in patients with CF are well characterized and relatively easy to measure. Correction of the abnormality would indicate that administration of the recombinant virus has corrected the CF electrolyte transport defect in the nasal epithelium.
Methods: Measurement of the nasal Vt is safe, easy, and not invasive (12,24). The Vt across the nasal epithelium will be measured using techniques similar to those previously described (12,41). A 23 gauge subcutaneous needle connected with sterile normal saline solution to a silver/silver chloride pellet (E.W. Wright, Guilford, CT) is used as a reference electrode. The exploring electrode is a size 8 rubber catheter (modified Argyle® Foley catheter, St Louis, MO) with one side hole at the tip. The catheter is filled with Ringer's solution containing (in mM), 135 NaCl, 2.4 KH_2PO_2, K_2HPO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$, and 10 Hepes (titrated to pH 7.4 with NaOH) and is connected to a silver/silver chloride pellet. Voltage will be measured with a voltmeter (Keithley Instruments INC., Cleveland, OH) connected to a strip chart recorder (Servocorder, Watanabe Instruments, Japan). Prior to the measurements, the silver /silver chloride pellets are connected in series with the Ringer's solution; the pellets are changed if the recorded Vt is greater than ±4 mV. The rubber catheter is introduced into the nostril under telescopic guidance (Hopkins Telescope, Karl Storz, Tuttlingen West Germany) and the side hole of the catheter is placed next to the inferior nasal turbinate. Basal Vt will be recorded until no changes in Vt are observed after slow intermittent 100 µl/min infusion of the Ringer's solution. Once a stable baseline is achieved, 200 µl of a Ringer's solution containing 100 µM amiloride (Merck and Co. Inc., West Point, PA) will be instilled through the catheter and changes in Vt will be recorded until no further changes are observed after intermittent installations. Finally, 200 µl Ringer's solution containing 100 µM amiloride plus 10 µM terbutaline (Geigy Pharmaceuticals, Ardsley, NY) will be instilled and the changes in Vt recorded. The entire procedure will take between 15-30 minutes.

Mucosal fluid for adenovirus antibody.
Purpose: To test for the presence on the mucosal surface of IgA or IgG antibody to the recombinant adenoviruses (types 2 and 5) and to test for neutralizing antibodies to these serotypes.
Methods: We will obtain a washing of the nasal surface to test for the presence of specific and neutralizing antibody.

Pulmonary function tests.
Purpose: To assess patient's pulmonary function.
Methods: Spirometry, lung volumes, and diffusing capacity will be measured in the Pulmonary Function Laboratory at the University of Iowa Hospitals and Clinics using standard techniques.

Arterial blood gases.
Purpose: To assess patient's pulmonary function.
Methods: Blood will be obtained by radial artery puncture and PO_2, PCO_2, and pH will be measured in the Pulmonary Function Laboratory at the University of Iowa Hospitals and Clinics.

PART A

Day relative to virus	-21 to -32	-7 to -14	First Administration 0	1	3	5	7	14	21	Second Through Fourth Administrations 0	1	3	5	7	14	21	Fifth Administration 0	1	3	5	7	14	21
Vector administration			1							2							5						
History and physical	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Blood samples																							
CBC	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
General screen	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
PT/PTT	•		•														•						
Serology	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Geneotyping	•																						
Bacterial cultures																							
Sputum	•		•							•							•						
Nasal swab	•																						
Viral culture on 293 cells																							
Nasal swab	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Pharynx	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Stool	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Sinus	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Urine	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Pulmonary function tests	•		•							•							•						
Arterial blood gases	•		•							•							•						
Chest CT	•																					•	
Nasal swab																							
Cell count and cytology	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Nasal Wash																							
Cytokines	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Serology	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Nasal voltage	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

High resolution computerized tomography of the chest.
Purpose: To assess patient's pulmonary disease.
Methods: High resolution, thin section computerized tomography of the chest will be performed in the Department of Radiology at the University of Iowa Hospitals and Clinics.

Biopsy of the nasal epithelium.
Purpose: To assess nasal mucosa and submucosa for evidence of an inflammatory/immune response.
Methods: After mild sedation, the nasal mucosa will be anesthetized by atomizing 0.5 ml of 5% cocaine. After endoscopic inspection, and identification of the site to be biopsied, the submucosa will be injected with 1% xylocaine, with 1/100,000 epinephrine. A small area of the mucosa of the inferior turbinate will be removed. After biopsy, the mucosa in the area of the inferior turbinate may be packed with cotton pledgets previously soaked in a mixture of 2 ml of 0.1% adrenaline and 8 ml of 1% tetracaine. The pledgets remained in place for 10-40 min. The biopsy will be performed on day 1, day 3, or day 7 after the last treatment. The first and fourth patient will be biopsied on day 1; the second and fifth patient will be biopsied on day 3; etc.

Potential Alterations in the Timing of the Procedures and the Doses.
The planned timing of the procedures and dose of Ad2-ORF6/PGK-CFTR are given above and in the flow chart. The biopsy will be performed after the last administration of vector. These timings and doses may, however, be changed by the investigators, based on the data. For example, if we observe convincing evidence of an inflammatory response by inspection of the nasal epithelium, by assessment of the cytology of the swabs of nasal mucosa, or by a systemic response, we will not proceed further, but instead will immediately proceed to a biopsy of the nasal mucosa on both the treated and the sham side. In the case that we see inflammation on the treated side, we will also alter the dosing strategy. As an example, suppose that we observe an inflammatory response after the third administration ($2x10^9$ IU). In the next patient, we would proceed with the first and second doses ($2x10^7$ IU and $2x10^8$ IU), but then for the third dose we would repeat the dose of $2x10^8$ IU. If there were no adverse effects we would then proceed with dose escalation.

Interpretation and Advantages.
This portion of the study focuses on the safety of an adenovirus vector - one of the most pressing issues in the use of adenovirus vectors for the treatment of CF lung disease - and its design provides several advantages in evaluating safety. These include the following:

a) Most importantly, because we use the nasal epithelium, we can obtain critical data about safety, yet we minimize any possible risk to the participants.
b) The quality of the control (the opposite nasal mucosa) is excellent. In each individual patient, the virus will be administered in a random manner to one of two nasal cavities and thereafter to the same cavity. All other systemic factors will be identical.
c) We will be able to assess biochemical efficacy by measuring the voltage across the nasal epithelium.

The results of these studies are critical to the design of future studies directed at delivering the gene to the pulmonary airways and treatment of the lung disease and to the design and development of future vectors (which may or may not be based on adenovirus) to treat CF.

Potential Risks Associated with the Virus.
The risks associated with somatic cell gene transfer using the Ad2-ORF6/PGK-CFTR gene construct in humans are unknown. However, we now have experience with administration of the related Ad2/CFTR-1 construct to humans (Appendix 7.8). The data suggest that there was no viral replication nor any adverse symptoms or findings that could be attributed to the recombinant virus.

Inflammation and Immune Response. It is possible that inflammation locally at the site of application of the virus could occur as a result of an immune response to the recombinant virus. Local cytopathic effects on the respiratory nasal epithelium are also possible. Inflammation or cytopathic effects include local pain, bleeding or subsequent scarring; although serious effects appear very unlikely. The use of nasal mucosa, rather than bronchial mucosa, is a major safety feature that should allow us to assess safety without endangering the patient.

Virus Replication. The Ad2-ORF6/PGK-CFTR viral construct has been rendered defective for replication by deletion of two important early genes E1a and E1b. The majority of the E4 region has also been deleted. However, it is possible that the virus will have a limited ability to replicate in human cells. Furthermore, under certain circumstances, the viral defect could be complemented. Such circumstances include coinfection with wild-type adenovirus or provision of E1 gene function by latent or residual adenovirus resulting from an earlier infection. Finally, epithelia might also provide normal cellular proteins with E1-like functions that are able to complement the defective virus.

Most of these possibilities seem remote. The likelihood of coinfection of treated cells with wild-type virus is minimized by prior screening of the patients and by treating only seropositive patients. Moreover, in the unlikely event that coinfection with wild type adenovirus did occur, Ad2-ORF6/PGK-CFTR is missing much of the E4 region and is unlikely to compete with wild-type virus. Thus, a wild-type infection would probably become self-limiting.

There is also a possibility of recombination between wild-type virus and Ad2-ORF6/PGK-CFTR. Whether this occurred by legitimate or illegitimate recombination, three separate events would be required to produce replication competent progeny virus: first, insertion of the missing E1 gene, second, insertion of E4 sequences, third, deletion of DNA sequences to enable the resulting recombinant to become small enough to package its DNA. The most likely sequences to be deleted, compatible with retention of biological activity, would be some or all of the CFTR DNA. Growth of such a recombinant would probably be self-limiting and would be unlikely to lead to synthesis of CFTR fragments with any significant biological activity.

Environmental Risks. There is a risk that the virus could be passed to another person. This could occur if a caregiver or visitor is exposed to the virus we apply to the patient or exposed to virus that has replicated in the patient. As described immediately above, replication of Ad2-ORF6/PGK-CFTR in the patient is very unlikely. To minimize the risk of exposure to persons other than the participant, the patient will be kept in isolation for one day after vector administration. In addition, we are using relatively small amounts of the virus which will limit the risk that others will be exposed to the virus. Were environmental release to occur, the most likely virus involved would be wild-type adenovirus that had overgrown Ad2/PGK-CFTR-1 or a recombinant with no biological activity other than that associated with wild-type virus.

Effects of Wild-type Adenovirus 2 Infection. Potential effects of wild-type adenovirus 2 include symptoms of upper respiratory infection. Pharyngitis and conjunctivitis are known to be associated with adenovirus. Strains of adenovirus other than adenovirus 2 have been known to cause pneumonia, transient diarrhea and gastroenteritis. Rare problems associated with adenoviral strains include cystitis and keratoconjunctivitis. Such problems may be hazardous in immunocompromised hosts. The possible role of adenovirus in oncogenesis has been under investigation with no evidence to suggest a role for adenovirus in human oncogenesis.

Potential Risks Associated with the Study Procedures.

Nasal and Pharyngeal Swabs. Nasal and pharyngeal swabs can produce mild discomfort when they are taken, but there is no serious or long term risk. They are standard practices in clinical medicine.

Biopsy of the Nasal Mucosa. Biopsy of the nasal epithelium will produce mild to moderate pain and discomfort. Pain and discomfort during the procedure will be minimized by use of local anesthesia. Subsequent use of analgesics will be allowed if needed. A small risk of bleeding or subsequent scar formation after the biopsy is possible as is expected for such a routine procedure. There is also a risk of infection. We will treat pain with analgesics and observe the area for bleeding or infection; if either should require it, we will administer appropriate treatment.

Venipuncture. Venipuncture to obtain blood samples produces minimal risks that are well-known.

Arterial Puncture. Arterial puncture includes the risk of bleeding, bruise formation, and pain at the time of the procedure. The procedure is standard and done frequently on outpatients.

Pulmonary Function Tests. Pulmonary function studies are routine on outpatients with a variety of lung diseases. There are no anticipated short or long term risks.

Measurement of Nasal Voltage. Measurement of Vt will not produce significant discomfort; it has generally been well tolerated in the past. The drugs applied topically to the nasal epithelium during the course of the study have no significant local or systemic effects.

Application of Ad2-ORF6/PGK-CFTR. Application of the recombinant virus to the nasal mucosa should have no significant risk. There could be minor discomfort or a sense of nasal obstruction. There may also be discomfort from having to remain still during the procedure. These discomforts should be minimized by administration of Midazolam 1-2 mg. IV. shortly before application. The major risks of Midazolam would be hypersensitivity which is very rare. The Midazolam could theoretically produce some respiratory depression; however the dose we will use should have no significant effects, particularly in these subjects who will only have mild to moderately severe disease.

Chest Computed Tomography Chest CT carries the risk of radiation exposure; we anticipate that a subject will have two chest CTs during the course of the study. Although there are no known adverse effects of this amount of radiation, the long term effects of such radiation are not known with certainty.

Potential Benefits.
It is very unlikely that participation will directly benefit the patient. The correction of the genetic defect of CF will likely be limited to the area of virus application in the nose for those patients enrolled in Part A and in the sinus for those patients enrolled in Part B.

The primary benefit will be the information obtained from this study regarding the safety and efficacy of gene transfer by recombinant adenovirus. Such information is critical for further development of gene transfer for CF lung disease. If this work is able to lead to the development of better treatments for CF, there will be a potentially large benefit to mankind.

The overall risks to the patients appear to be small in view of the care taken to develop a viral/gene construct which is defective in replication and which, in wild-type form, is known to produce relatively mild disease. The risks are minimized by use of nasal mucosa. Given the potential benefits of the information to be gained by human trials, the risk appears to be justified.

STUDY PLAN, PART B

Study Design.
This is a non-randomized, nonblinded administration of recombinant Ad2-ORF6/PGK-CFTR to the maxillary sinus epithelium. Although administration of vector will not be blinded, the analysis of many of the results will be blinded, using the saline-treated contralateral maxillary sinus as a control.

Number of Participants.
We intend to study five to ten patients. All patients will be identified in the first year. Patients will be referred to the study for consideration their physicians.

Selection Criteria.
Inclusion Criteria
The criteria are the same as the selection criteria in Part A with the following additional criterion:
e) Maxillary sinus disease. Patients must have bilaterally opacified sinuses on sinus CT scan.

Exclusion Criteria
The criteria are the same as the exclusion criteria in Part A with the following additional criterion:
m) Patients would be excluded from the study if the CT scan demonstrated sinuses that were so hypoplastic that there would be potential technical difficulties in their safe cannulation. The patient's nose would be endoscopically examined and those patients with massive polyposis or with severe septal deformities would be excluded. Those two factors would serve to prevent proper anatomical access to the maxillary sinus via the inferior meatus.

Timing of Evaluations and of Repeat Administrations.
Timing of pretreatment evaluations and repeat administrations are the same as in Part A of this protocol. The flow sheet shows the timing of specific interventions and evaluations.

Timing of Initiation of Study.
We will not begin Part B of this study until we have administered a dose of $2x10^9$ IU to at least two patients in Part A. We will also use the data from Part A to guide us in Part B. For example, if we find evidence of a response to Ad2-ORF6/PGK-CFTR that would suggest problems with safety, we will delay Part B until the source of the problems can be identified and solved.

Vector Application to the Maxillary Sinus.
Patients will be admitted to the Clinical Research Center at the University of Iowa. After vector administration, they will be placed in isolation in a hospital room for one day. They will be discharged from the hospital the day after Ad2-ORF6/PGK-CFTR administration.

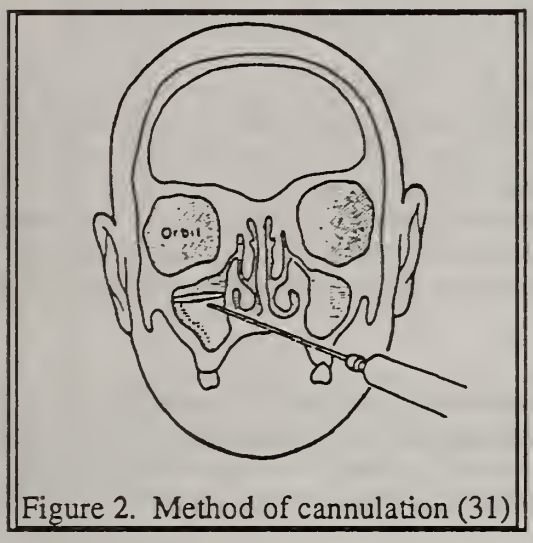
Figure 2. Method of cannulation (31)

The patient's nose will initially be suctioned free of any excess mucus. It will then be sprayed with a solution of 2% Pontocaine and 1/4% Neo-Synephrine. In addition, as mild sedation and to minimize movement during the application, they will also receive Midazolam 1-2 mg. IV. shortly before application. Five min after spraying, the nose will be endoscopically examined. At this stage, under endoscopic control, cottonoid neurosurgical pledgets soaked in a solution of 5% cocaine (to a maximal dose of 100 mg) will be placed high in the inferior meatus. After 15 min the pledgets will be removed. The inferior meatus will be visualized using a headlight and a Lichtwitz cannula introduced into the maxillary sinus via the inferior meatus (Fig. 2). This cannula is introduced high in the inferior meatus near the apex of the attachment of the inferior turbinate in the thin bone and membranous portion of the lateral nasal wall (42,43). This procedure is a very common, simple outpatient procedure in the Otolaryngology Clinic.

Fluid will be removed from the maxillary sinuses and evaluated for: a) quantitative bacteriologic cultures (both aerobic and anaerobic); b) cytologic analysis for total and differential white blood count; c) evaluation of inflammatory cytokines and mediators including IL-1ß, IL-8, and TNF (44-46); these agents are associated with inflammatory airway disease in CF; d) type-specific and neutralizing antibody to adenovirus. The maxillary sinuses will then be lavaged with saline several times and we will measure transepithelial voltage (V_t). Although we have not previously measured V_t in the maxillary sinuses, we will use methodology similar to that which we use in the nasal epithelium.

After completion of these procedures, both maxillary sinuses will be cannulated with indwelling Silastic flanged tubes. The soft flanges will maintain the tubes in the sinuses. The other end of the tube will be taped to the cheek. Maxillary sinus lavage with antibiotics will be continued three times a day for seven days. The choice of antibiotics will initially be guided by previous cultures from the patient and then by results of our cultures from the maxillary sinuses. For example, lavage with 40 mg tobramycin three times a day is frequently used (30). After such treatment, previous studies have shown that the maxillary sinuses are relatively free of mucus and thus should be accessible to the applied vector. Of importance, even with such treatment, the mucosa will remain thickened and the secretions invariably reaccumulate. On the 8th day, we will repeat the lavage and make the measurements described above, including a limited-cut CT of the maxillary sinus. Then the vector will be applied and the catheters will be removed.

At the time of repeat vector administration, we will repeat all of the evaluations (including the limited-cut maxillary sinus CT, measurement of sinus V_t, cultures, measurement of inflammatory cells, cytokines, etc., see below). We will administer the vector using the same procedures as described for the first administration. We will not, however, place the indwelling Silastic flanged tubes nor will we lavage the

sinuses for one week. Instead, we will enter the sinus, rinse it free of mucus and debris, and instill the vector.

Dose of Vector.
Each participant will receive the same doses of Ad2-ORF6/PGK-CFTR:

Administration	Total IU
1	$5x10^8$
2	$2.5x10^9$
3	$2.5x10^9$
4	$2.5x10^9$
5	$2.5x10^9$
6	$2.5x10^9$
7	$2.5x10^9$

Number of Repeat Administrations.
In this part of the study, we plan to administer Ad2-ORF6/PGK-CFTR a maximum of 7 times. If we see evidence of clear improvement or clear worsening on the treated as compared to the control maxillary sinus, we will decrease the total number of administrations. Evidence of improvement or worsening would be obtained from two consecutive CT scans that showed a clear difference between the treated and control sinuses. The cytology, cultures, and cytokines measured in sinus fluid will also be evaluated. Alternatively, we would shorten the study if we obtain evidence of a systemic or local inflammatory response. At the completion of the study we will perform a biopsy of both sinuses to obtain histologic evidence of improvement or of injury.

List of Evaluation Procedures.
The following evaluation procedures will be performed as described in the Study Plan of Part A:
a) History and Physical Examination; b)Blood/Serum by Venipuncture; c)Viral Cultures of Nasal and Pharyngeal Swabs, Urine and Stools; d) Swab of nasal mucosa for evaluation of an inflammatory response; e) Pulmonary Function Tests; f) Arterial Blood Gases; g) Voltage across the sinus epithelium.
The following evaluations are specific to Part B:

Evaluation of Fluid from Maxillary Sinuses.
Purpose: To assess the severity of maxillary sinus disease.
Methods: Fluid obtained at the time of initial sinus cannulation and then at the time of sinus lavage will be evaluated for quantitative bacterial cultures, cytology, cell counts, and cytokines and mediators including IL-1ß, IL-8, and TNF (44-46). It will also be evaluated for antibodies to adenovirus as described in part A.

Computerized Tomography (CT) of the Sinuses.
Purpose: To assess maxillary sinus disease.
Methods: Computerized tomography (CT) of the sinuses will be performed at enrollment into the study. Subsequently, limited-cut CT will be performed, unless indicated by a change in clinical status. We will perform limited-cut maxillary sinus CT to minimize radiation exposure, yet still obtain a high resolution image for evaluation. Sinus CT will be performed in the Department of Radiology at the University of Iowa Hospitals and Clinics. Films will be evaluated in a blinded manner by Dr. Scott Graham and by physicians in the Department of Radiology.

PART B

Day relative to virus admin	First Administration														Subsequent administrations					
	-21	-7	-6	-5	-4	-3	-2	-1	0	1	3	7	14	21	0	1	3	7	14	21
History	•	•							•						•					
Physical exam	•	•							•						•					
Limited Hx & Px			•	•	•	•	•	•		•	•	•	•	•		•	•	•	•	•
Blood samples																				
CBC	•	•			•				•	•	•	•	•	•	•	•	•	•	•	•
General screen	•	•			•				•	•	•	•	•	•	•	•	•	•	•	•
PT/PTT	•	•							•						•					
Serology	•	•							•	•	•	•	•	•	•	•	•	•	•	•
Genotype	•																			
Viral cultures on 293 cells																				
Nasal swab	•	•							•	•	•	•	•	•	•	•	•	•	•	•
Pharynx	•	•							•	•	•	•	•	•	•	•	•	•	•	•
Stool	•	•							•	•	•	•	•	•	•	•	•	•	•	•
urine	•	•							•	•	•	•	•	•	•	•	•	•	•	•
Sinus	•	•							•	•	•	•	•	•	•	•	•	•	•	•
Bacterial cultures																				
Sputum	•																			
Sinus, quant.		•							•											
Sinus fluid																				
Cytology & cell count		•							•						•					
cytokines		•							•						•					
PFTs																				
Complete	•																			
Spirometry		•							•						•					
ABGs	•	•							•						•					
Sinus CT	•																			
Sinus CT, limited-cut									•						•					
Nasal voltage	•	•																		
Sinus voltage		•							•						•					
Virus administration									•						•					
Inpatient days		•	•	•	•	•	•	•	•	•					•	•				
Lavage sinus c antibiotics		•	•	•	•	•	•	•												
IV antibiotics		•	•	•	•	•	•	•												

Maxillary Sinus Mucosa Biopsy.
Purpose: To determine if there was: a) biochemical efficacy (by RT-PCR, by *in situ* hybridization, and by immunocytochemistry); b) clinical efficacy (by histologic evaluation of the mucosa and submucosa for inflammation characteristic of CF); and c) another inflammatory or immune response induced by virus application (by histologic evaluation and immunocytochemistry of inflammatory cells). There is no definitive way to distinguish between an inflammatory response resulting from CF and chronic infection, and an inflammatory response secondary to an immune response to the vector. However, we may find a histologically different picture in the two situations. For example, if in the treated mucosa there is a predominance of mononuclear cells and lymphocytes and the presence of CD4 or CD8 positive cells that is different from that in the control mucosa, then we would conclude that vector administration produced inflammation.
Methods: The patient will be sedated with intravenous midaxolam and meperidine, titrated to patient response. The patient will be continuously monitored with EKG, pulse oximetry and intermittent automatic blood pressure assessment. The upper gingivo-buccal sulcus will be infiltrated with 4 ml of 1% xylocaine with 1/100,000 epinephrine, with injection of the underlying periosteum. After 10 min a 2.5 cm sublabial incision is made and a subperiostial flap elevated to expose the canine fossa. Once the canine fossa is identified a medium cutting burr will initially be used to drill away the cortical bone over a 5 mm area. A diamond burr will then be used to remove the remaining bone and expose the underlying maxillary sinus mucosa over the same 5 mm diameter area. The mucosa will be sharply excised to obtain a biopsy specimen. The interior of the sinus will be inspected and photographed endoscopically, and V_t measured. Hemostasis will be monitored and observed until the patient is fully awake.

Interpretation and Advantages.
These studies should provide data about the potential for clinical efficacy of adenovirus-directed transfer of CFTR in treating CF airway disease. This part of the study has several advantages.

a) The quality of the control (the opposite maxillary sinus) is excellent. Virus will be administered to one of two maxillary sinuses: otherwise both sinuses will be treated identically. All other systemic factors, such as administration of antibiotics for pulmonary exacerbations, will be identical for both maxillary sinuses and should have little appreciable effect on the course of sinus disease.
b) Adenovirus has the potential to cause an inflammatory/immune response on its own. Because this study will measure clinical efficacy, which is the net beneficial effect of expressing CFTR and the potential adverse effects associated with administration of vector, our results should give an indication of the net balance between the benefits and the risks.
c) If vector administration does not prove clinically efficacious, we will be able to determine whether the lack of clinical efficacy resulted from a failure to express CFTR.
d) We should be able to assess clinical efficacy in a relatively short period of time without entailing the risks and potential complications of repeated bronchoscopy.
e) Studies of the maxillary sinus epithelium should provide us with an assessment of safety and clinical efficacy while minimizing risk to participants.

The results of these studies are critical to: the design of future studies in larger numbers of patients; the design of future studies directed at delivering the gene to the pulmonary airways and treatment of lung disease; and the design and development of future vectors to treat CF.

Potential Risks Associated with the Virus.

The potential risks associated with the virus are described in detail in Part A.

Potential Risks Associated with the Study Procedures.

The potential risks associated study procedures common to Parts A and B are described in Part A. The following are the potential risks associated with study procedures that are specific to Part B.

Measurement of Voltage Across the Sinus Epithelium.
Measurement of the transepithelial electrical potential difference across the sinus epithelium should not produce significant discomfort; it is well tolerated in the nasal epithelium. The drugs applied topically during the course of the study have no significant local or systemic effects.

Application of Ad2-ORF6/PGK-CFTR.
Placement of the indwelling catheters in the maxillary sinuses 7 days before vector administration could produce discomfort. There may also be minor discomfort from having to remain still during the procedure. These discomforts should be minimized by local anesthesia with topical 2% lidocaine to the nasal mucosa and by administration of Midazolam 1-2 mg. IM shortly before application. The major risks of lidocaine and Midazolam would be hypersensitivity which is very rare. The Midazolam could theoretically produce some respiratory depression; however the dose we will use should have no significant effects, particularly in these subjects who will only have mild to moderately severe disease. There is also the risk of minor bleeding at the site of entry into the maxillary sinus. Lavage of the maxillary sinuses could cause discomfort. On subsequent application of virus the participants may experience similar discomfort. During the week before the first administration of vector the participants will be treated with IV antibiotics. The specific antibiotics will be chosen based on the culture and sensitivities of the sputum and nasal/pharyngeal swabs. We will monitor blood levels of antibiotics and adjust the doses appropriately. Such treatment is common for patients with CF.

Biopsy of the Maxillary Sinus Mucosa.
Biopsy of the maxillary sinus epithelium will produce mild to moderate pain and discomfort. Pain and discomfort during the procedure will be minimized by use of local anesthesia. Subsequent use of analgesics will be allowed if needed. A small risk of bleeding or subsequent scar formation after the biopsy is possible as is expected for such a routine procedure.

Sinus Computed Tomography.
Sinus CT carries the risk of radiation exposure; we anticipate that a subject will have one sinus CT, and up to 8 limited cut CTs of the maxillary sinuses during the course of the studies. Although there are no known adverse effects for this amount of radiation, the long term effects of such radiation are not known with certainty.

Potential Benefits.
The potential benefits are described in Part A of this protocol.

OTHER ISSUES

Confidentiality.
Confidentiality will be maintained throughout the study. Publication of results will not identify the patients. Genzyme Corporation agrees to maintain confidentiality.

Compensation to Participants.
Participants will be compensated for the time and inconvenience involved in participating in the research in the amount of $75 for each day spent in the hospital. In addition, we will reimburse them for their travel expenses to and from the University hospitals at a rate of $0.25 per mile and for parking expenses. No additional costs to the subject are anticipated as a result of participation in the study.

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CERTIFICATION OF SUBJECT CONSENT

Project Title: **Adenovirus-mediated gene transfer for cystic fibrosis: Safety of dose and repeat administration in the nasal epithelium**

Investigators: Michael J. Welsh, M.D.
Joseph Zabner, M.D.
Scott M. Graham, M.D.

I, _____, hereby certify that I have
(subject's name)

been told by Dr. Michael Welsh or Dr. Joseph Zabner of The University of Iowa Medical Center about research on gene therapy for cystic fibrosis and its purposes. I have been told about the procedures to be followed, which of them are experimental, and how much time is involved. I understand the possible risks and discomforts I may experience and the possible benefits to me and to others from the research. I have also been told the extent to which any records which may identify me will be kept confidential.

A written summary of what I have been told is attached. I have been given adequate opportunity to read it.

I understand that I have the right to ask questions at any time and that I should contact Dr. Michael Welsh (319) 335-7619 or Dr. Joseph Zabner (319) 335-7574 for answers about the research and my rights.

I understand that in the event of physical injury resulting directly from the research procedures no compensation will be available in the absence of negligence by a state employee. However, medical treatment is available at the University Hospitals and Clinics, but I will be responsible for making arrangements for payment of the expenses of such treatments. Further information may be obtained from Dorothy M. Maher, Division of Sponsored Programs, Office of the Vice President for Research, (319) 335-2123.

I understand that my participation is voluntary, that I may refuse to participate or withdraw my consent for experimental studies at any time without penalty or loss of benefits to which I may be entitled.

I hereby freely consent to take part in this research project.

_____ _____
Signature of Subject Date

I, the undersigned, certify that I was present during the oral presentation of the written summary attached, when it was given to the above subject.

_____ _____
Signature of Auditor-witness Date

INFORMATION SUMMARY FOR

INFORMED CONSENT

TITLE: **Adenovirus-mediated gene transfer for cystic fibrosis: Safety of dose and repeat administration in the nasal epithelium**

INVESTIGATORS: Michael J. Welsh, M.D.
Joseph Zabner, M.D.
Scott M. Graham, M.D.

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1. INTRODUCTION AND PROCEDURES:

During the course of treatment of your disease, you have learned that cystic fibrosis is a serious genetic disease that causes the airways of the lung to fill with thick mucus. This situation leads to frequent infections which in turn cause destructive changes in the lung and increasing problems with breathing. The basic problem is a defect in a gene that is responsible for producing a protein called the cystic fibrosis transmembrane conductance regulator or CFTR. This protein regulates salt balance in the airways of the lungs; when the protein is not normal, excessive thick mucus is produced. Antibiotics have been helpful and are used to treat the infections that result from this problem. Various ways to clear the mucus, such as chest physical therapy, are also useful to treat cystic fibrosis. However, none of these treatments correct the basic problem. Thus, despite all efforts, the problem in the lungs usually worsens with time.

1a. This study involves research in gene therapy for cystic fibrosis. The ultimate purpose of the research is to find a better treatment for cystic fibrosis. In this study we will examine the safety and possibility of correcting the basic genetic defect by applying an altered virus onto the cells of the airway in people who have cystic fibrosis.

1b. You are being invited to participate in this research because you have cystic fibrosis and because your disease is of mild to moderate severity. It is unlikely that participation in this study will be of any benefit to you. After enrolling into the study you will be monitored for a period of at least three weeks before the treatment. You will be admitted to the hospital on the day of the treatment and discharged on the following day. You will need to return to the clinic for an evaluation 3, 7, 14, and 21 days after the treatment. The treatment will be repeated five times, with at least 4 weeks between each treatment. After each treatment you will need to stay in the hospital for one day and then return to the clinic at the same time intervals as you do after the first treatment.

1c. If you agree to participate, these procedures will be followed.

An extensive evaluation will be performed that includes a complete physical exam, computed tomographic X-rays of your chest and pulmonary function tests. Blood will be drawn from your vein and artery (total of about 1 cup) for multiple studies. We will also determine whether or not you have immunity to adenovirus, so that we know that you already have protection against the virus. By collecting specimens of nasal secretions, stool, and urine, we will also determine that you do not already have the natural type of adenovirus in your body. The specific genes for cystic fibrosis which you carry in your chromosomes will be determined from a blood sample. We will also measure the voltage that normally exists across the cells that line your nose. You will be evaluated with these tests when you enter the study. You will then be clinically evaluated at about ten days

before you are given the altered virus. The wait from the time of enrollment into the study and administration of the altered virus will be at least three weeks, but could be significantly longer. Most of the tests will be repeated just before you are given the altered virus, and some will be repeated during the visits between enrollment into the study and administration of the virus.

The virus, known as adenovirus 2, is a common virus that is found in human airways and can cause a "cold". The virus that you will receive has been altered so that it cannot reproduce normally. These changes limit its ability to cause disease, to grow, and to spread. The normal gene for the cystic fibrosis protein, CFTR, has been inserted into the virus. In the laboratory, it has been shown that this altered virus can enter the cells that form the lining of the human nose and deposit the normal gene, so that normal CFTR is produced by the cells. Studies with laboratory animals suggest that the altered virus does not cause disease or illness. They also indicate that the virus can carry a new gene into the cells of the airway. However, there is no guarantee that the virus will behave the same way in humans.

The day the virus is applied, you will be admitted to the hospital. The virus you will receive has been prepared at Genzyme Corporation in Framingham, Massachusetts. To put the virus into the lining of the nose, we will use a small tube to place a few drops into the nose on one side. On the other side, we will place a few drops of a salt solution, for purposes of comparison. The application procedure will last about 30 or 40 minutes. We will give you a mild tranquilizer that will reduce anxiety and help you sit still. Each time we repeat the treatment we will use a higher dose of the virus. In this way we can learn if the virus is safe. We plan to administer the virus five times at approximately one month intervals. We expect that the entire study will take about six months. This procedure is experimental.

To determine whether the virus carrying CFTR is safe and can correct the cystic fibrosis respiratory abnormalities, we will perform several studies on days 1, 3, 7, 14 and 21 after the treatment. We will take swabs from both nostrils and your throat. The nasal and throat swabs are performed by gently rubbing a cotton-tipped applicator in the inside of your nose and in your throat. These procedures are commonly performed to diagnose "strep throat" and are not harmful or painful.

Because this is an experimental virus, there is a possibility that inflammation of the lining of the nose may occur. It is possible that redness and pain could occur or that you could develop symptoms similar to those experienced when you have a cold: that is a stuffy or runny nose. To determine if any harm was done to the area of nose where the virus was given, we will perform a nasal biopsy. Prior to the biopsy, an anesthetic to numb the feeling in the area will be used. A small area (about half the size of a dime) will be removed from the lining of both sides of your nose by an Ear, Nose and Throat doctor. This will be done after the completion of all the treatments or earlier if we find any evidence of injury from the virus.

To learn whether the experimental virus can correct cystic fibrosis respiratory abnormalities, we will measure the voltage across the lining of the nose. This involves the insertion of a needle into the skin on your arm, and touching the inner surface of the nose with a small rubber catheter. The rubber catheter and the needle will be connected to a voltmeter. A voltmeter is a recording device and does not deliver any electricity to your nose. Two different drugs at very low doses will be perfused onto the lining of the nose for approximately 30 minutes. These drugs are terbutaline (which is used to treat asthma), and amiloride (which is used to treat heart and kidney problems). These drugs

are approved for human use, and at the low concentration used, do not have major side-effects.

After application of the virus you will be required to be in respiratory isolation for one day. We wish to keep you in isolation so that no one else will be exposed to the virus. This means that you will be confined to a hospital room and visitors will be required to wear protective masks, gloves, and gowns.

About 2 tablespoons of blood will be drawn on the day of admission and discharge and on days 1, 3, 7, 14, and 21. Additional blood samples will be required with the additional four treatments. Computerized tomographic X-rays of your chest, and repeat pulmonary function tests will be performed at the end of the study or if there is a change in your disease.

2. **FORESEEABLE RISKS AND DISCOMFORTS:**

Risks associated with gene transfer using the altered virus are largely unknown. It is possible that the virus could cause an inflammation locally at the site of application in the nose or it could damage the tissue in the nose. These problems might produce local pain or bleeding in the nose. There is also the very unlikely possibility of scarring in the nose. It is possible that the virus might reproduce under certain circumstances; this unlikely possibility might lead to spread of the virus and symptoms of a "cold". This may include nasal congestion, runny nose, sneezing, cough or fever. It is possible that a pneumonia could occur but this is very unlikely because we will only apply the altered virus to the lining of the nose. Other symptoms caused by naturally occurring adenoviruses include sore throat and red, sore eyes. Adenovirus that occurs naturally has been known to cause diarrhea and pneumonia, though these usually are caused by different types of adenovirus than that which was prepared for this study. If a lung infection or pneumonia did somehow occur, then it might cause a worsening of your symptoms of cystic fibrosis. Rare problems caused by adenovirus include an inflammation of the bladder. An extremely unlikely possibility is the formation of a new type of virus through interaction with other viruses. It is possible that participation in the study could cause your immune system to form a response against the altered virus. This could affect your response to subsequent treatments with the altered virus. It is also possible that you may develop an allergic reaction to the virus. The virus you will be exposed to, rarely introduces new gene into chromosomes. This makes it exceedingly unlikely that the altered virus could cause cancer. It also means that correction of the genetic defect in the lining of your nose will be temporary.

The method for application of the virus may be uncomfortable, you will be required to sit still for about 30 to 40 minutes without moving your head. Placement of the small rubber tube in your nose could induce itching and sneezing. It is unlikely that the actual placement of the virus on the lining of the nose will cause significant discomfort.

The biopsy of the nasal mucosa may be painful and a small amount of bleeding will be expected (about 2 tablespoons). After the biopsy, the healing process takes about 2 weeks and some pain, itching, nasal discharge, and minor bleeding are expected. Pain killers will be available as needed. There is a small risk of infection at the site of the biopsy.

The procedure involved in measuring the voltage on the inner surface of the nose does not cause pain and is unlikely to produce discomfort other than some itching. Two drugs will be applied to the lining of the nose; each is approved by the Food and Drug Administration for human use. The two drugs used are: terbutaline, which can

temporarily increase the heart rate and amiloride, which is a "fluid pill" medication when taken by mouth. At the very low doses that will be applied to the lining of the nose, these medications should not cause problems.

The drawing of blood from a vein or from an artery for blood tests could cause a bruise at the site, minor bleeding, and momentary pain.

We anticipate that a subject will have two computerized tomographic X-rays of the chest during the course of the studies. Although there are no known adverse effects of this amount of radiation, the long term effects of such radiation are not known with certainty. The risk associated with the radiation that you will receive from this study is approximately equal to one fifth of the annual allowable dose to a radiation worker.

The altered adenovirus, could be dangerous to an embryo or fetus if you are pregnant; the risks are currently unknown. You will not be allowed to participate if you are pregnant. If you are a woman, we will do a pregnancy test. You will be asked to adopt some form of contraceptive precaution, such as oral contraceptives or an intrauterine device, during this experiment.

During the time that you are participating in this study you will remain susceptible to the problems that are normally associated with cystic fibrosis, such as infections of the lungs. If during the course of this study, you should develop a complication of your cystic fibrosis, we would initiate the appropriate treatment; for example, if you should develop an pneumonia, we would begin treatment with antibiotics. However, in that case, we would ask that you or your insurance company arrange for payment for the therapy.

We do not expect that you will experience any major harmful effects of this study. However, because it could be of importance for future patients, an autopsy would be of value in determining the effects of the treatment. Therefore, in the unlikely event of death from any cause (for example, a traffic accident) during the course of the study, we will request that permission for an autopsy be granted.

3. **POTENTIAL BENEFITS TO YOU OR TO OTHERS WHICH MAY BE EXPECTED FROM THE RESEARCH:**

It is unlikely that participation in this study will directly benefit you. The correction of the genetic defect of cystic fibrosis will likely be limited to only the actual area where the virus was applied to the nose. The primary benefit will be a better understanding of the possibilities for gene therapy with the altered virus in cystic fibrosis. In particular, information regarding the amount of virus needed and the effects of the virus on the cells of the airway will be critical for further studies which might involve giving the virus to the bronchial airways of the lung to treat the disease.

4. **ALTERNATIVES THAT ARE AVAILABLE TO YOU:**

You may elect not to participate in this study and this decision will not affect your care at the University of Iowa. Treatments of cystic fibrosis include: chest physiotherapy, antibiotics, nutritional and dietary measures, and experimental agents which might reduce the thickness of the mucus. These treatments will also be available to you during and after the study. Since this study will not directly treat your lungs, a decision not to participate will not affect your health.

5. CONFIDENTIALITY:

A record of your participation in this research will be maintained, but this record will be kept confidential. You will be assigned a study number and information will be kept under the number rather than your name. All requests for information will be directed to the University of Iowa Health Science Relations Office to minimize the risk that your identity will be disclosed to the public. Gene therapy is very interesting to the general public. Although every effort will be made to maintain your privacy, it is possible that the news media or other individuals might learn your identify and spread this information, even if you do not want this to happen.

6. DISCLOSURE OF INFORMATION TO REGULATORY AGENCIES:

The U.S. Food and Drug Administration (FDA), National Institutes of Health, and Genzyme Corporation may inspect and copy your medical records relating to this study, and the results of the study will be reported to the sponsor, the FDA and perhaps to other regulatory agencies. This information will be treated confidentially and, in the event of any publication regarding this study, your identity will not be disclosed.

7. RIGHT TO ASK QUESTIONS:

Questions about this research will be answered by either Dr. Michael J. Welsh, M.D., University of Iowa Medical Center, telephone (319) 335-7619 or Dr. Joseph Zabner, MD., University of Iowa Medical Center, telephone (319) 335-7574.

8. RIGHT TO WITHDRAW:

Your participation in this study is voluntary. No penalty or loss of benefits to which you are entitled will occur if you decide not to participate. After enrollment in the study, you may discontinue participation at any time before application of the virus without penalty. Because you will receive a virus for which safety data in humans is not yet available, the risks are not yet fully defined. Therefore, if after having received the virus, you wish to withdraw from the study, you may do so but we request that you continue to return to the clinic for evaluation. If you feel that you are not able to make this commitment, we recommend that you not enter the study.

In addition, Dr. Welsh may terminate your participation in this study at any time he deems appropriate, after he has explained the reasons for doing so and has helped arrange for your continued care by your own physician if that is appropriate. Reasons that he might discontinue your participation could, for example, include a significant worsening of your clinical status unrelated to the experimental procedure.

9. COMPENSATION:

There will be no charge to you for participating in this study nor will you be charged for any tests that we perform. You will be compensated for the time and inconvenience involved in participating in the research in the amount of $75 for each day that you spend in the hospital. In addition, we will reimburse you for your travel expenses to and from the University Hospitals for each day that you come to the clinic for an outpatient visit. The rate will be $0.25 per mile and your parking expenses.

10. IOWA FAIR INFORMATION PRACTICES ACT

The Iowa Fair Information Practices Act (Section 22.11, Code of Iowa) requires that persons supplying information be notified of the use that will be made of the information, which persons outside the University might routinely be provided this information, which parts of the information requested are required and which are optional, and the consequences of failing to provide the information required.

I have discussed the above points, including the information required by the Iowa Fair Information Practices Act, with the subject or the legally authorized representative, using a translator when necessary. It is my opinion that the subject understands the risks, benefits, and obligations involved in participation in this project.

_____	_____	_____	_____
Investigator	Date	Auditor-Witness	Date

CERTIFICATION OF SUBJECT CONSENT

Project Title: **Adenovirus-mediated gene transfer for cystic fibrosis: Safety of repeat administration and clinical efficacy in the maxillary sinus**

Investigators: Michael J. Welsh, M.D.
Joseph Zabner, M.D.
Scott M. Graham, M.D.

I, _____, hereby certify that I have
(subject's name)

been told by Dr. Michael Welsh or Dr. Joseph Zabner of The University of Iowa Medical Center about research on gene therapy for cystic fibrosis and its purposes. I have been told about the procedures to be followed, which of them are experimental, and how much time is involved. I understand the possible risks and discomforts I may experience and the possible benefits to me and to others from the research. I have also been told the extent to which any records which may identify me will be kept confidential.

A written summary of what I have been told is attached. I have been given adequate opportunity to read it.

I understand that I have the right to ask questions at any time and that I should contact Dr. Michael Welsh (319) 335-7619 or Dr. Joseph Zabner (319) 335-7574 for answers about the research and my rights.

I understand that in the event of physical injury resulting directly from the research procedures no compensation will be available in the absence of negligence by a state employee. However, medical treatment is available at the University Hospitals and Clinics, but I will be responsible for making arrangements for payment of the expenses of such treatments. Further information may be obtained from Dorothy M. Maher, Division of Sponsored Programs, Office of the Vice President for Research, (319) 335-2123.

I understand that my participation is voluntary, that I may refuse to participate or withdraw my consent for experimental studies at any time without penalty or loss of benefits to which I may be entitled.

I hereby freely consent to take part in this research project.

_____ _____
Signature of Subject Date

I, the undersigned, certify that I was present during the oral presentation of the written summary attached, when it was given to the above subject.

_____ _____
Signature of Auditor-witness Date

INFORMATION SUMMARY FOR

INFORMED CONSENT

Project Title: **Adenovirus-mediated gene transfer for cystic fibrosis: Safety of repeat administration and clinical efficacy in the maxillary sinus**

Investigators: Michael J. Welsh, M.D.
Joseph Zabner, M.D.
Scott M. Graham, M.D.

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1. INTRODUCTION AND PROCEDURES:

During the course of treatment of your disease, you have learned that cystic fibrosis is a serious genetic disease that causes the airways of the lung to fill with thick mucus. This situation leads to frequent infections which in turn cause destructive changes in the lung and increasing problems with breathing. The basic problem is a defect in a gene that is responsible for producing a protein called the cystic fibrosis transmembrane conductance regulator or CFTR. This protein regulates salt balance in the airways of the lungs; when the protein is not normal, excessive thick mucus is produced. Antibiotics have been helpful and are used to treat the infections that result from this problem. Various ways to clear the mucus, such as chest physical therapy, are also useful to treat cystic fibrosis. However, none of these treatments correct the basic problem. Thus, despite all efforts, the problem in the lungs usually worsens with time.

1a. This study involves research in gene therapy for cystic fibrosis. The ultimate purpose of the research is to find a better treatment for cystic fibrosis. In this study we will examine the safety and possibility of correcting the basic genetic defect and the clinical abnormalities by applying an altered virus onto the cells of the airway in people who have cystic fibrosis.

1b. You are being invited to participate in this research because you have cystic fibrosis and because your disease is of mild to moderate severity. It is unlikely that participation in this study will be of any benefit to you. After enrolling into the study you will be monitored for a period of at least three weeks before the treatment. You will be admitted to the hospital for seven days before the first treatment, during which time you will be given antibiotics by vein and your maxillary sinuses will be washed three times a day with antibiotics. On the day after treatment you will be discharged from the hospital. You will need to return to the clinic for an evaluation 3, 7, 14, and 21 days after the treatment. The procedure will be repeated up to a maximum of six times (for a total of seven applications of the virus), with about 4 weeks between each treatment. After each treatment you will need to stay in the hospital for one day and then return to the clinic at the same time intervals as you do after the first treatment.

1c. If you agree to participate, these procedures will be followed.

An extensive evaluation will be performed that includes a complete physical exam, computed tomographic X-rays of your sinuses, and pulmonary function tests. Blood will be drawn from your vein and artery (total of about 1 cup) for multiple studies. We will also determine whether or not you have immunity to adenovirus, so that we know that

you already have protection against the virus. By collecting specimens of nasal secretions, stool, and urine, we will also determine that you do not already have the natural type of adenovirus in your body. The specific genes for cystic fibrosis which you carry in your chromosomes will be determined from a blood sample. We will also measure the voltage that normally exists across the cells that line your nose. You will be evaluated with these tests when you enter the study. You will then be clinically evaluated at about ten days before you are given the altered virus. The wait from the time of enrollment into the study and administration of the altered virus will be at least three weeks, but could be significantly longer. Most of the tests will be repeated just before you are given the altered virus, and some will be repeated during the visits between enrollment into the study and administration of the virus.

The virus, known as adenovirus 2, is a common virus that is found in human airways and can cause a "cold". The virus that you will receive has been altered so that it cannot reproduce normally. These changes limit its ability to cause disease, to grow, and to spread. The normal gene for the cystic fibrosis protein, CFTR, has been inserted into the virus. In the laboratory, it has been shown that this altered virus can enter the cells that form the lining of the human nose and deposit the normal gene, so that normal CFTR is produced by the cells. Studies with laboratory animals suggest that the altered virus does not cause disease or illness. They also indicate that the virus can carry a new gene into the cells of the airway. However, there is no guarantee that the virus will behave the same way in humans.

Seven days before the virus is applied, you will be admitted to the hospital. On that day, we will numb the interior of your nose and give you a mild tranquilizer that will reduce anxiety and help you sit still. We will place a small tube through your nostril into each maxillary sinus. The tube will be left in place for seven days, during which time we will use it wash the sinuses and to instill antibiotics into the sinuses. This will be done three times a day. We will also give you antibiotics by vein to treat your lung disease and disease in the sinuses.

On the eighth day after you are admitted, we will put a few drops of the altered virus into one of the sinuses. For purposes of comparison, in the other sinus we will place a few drops of a salt solution. The virus you will receive has been prepared at Genzyme Corporation in Framingham, Massachusetts. Then the tubes will be removed. You will be discharged from the hospital the next day. The treatment will be repeated up to a maximum of six times, for a total maximum of seven treatments. For the second through seventh treatments, you will be admitted to the hospital a small tube will be placed into the sinuses as described above, the sinuses will be washed, the virus will be inserted, and then the tubes will be withdrawn. You will be discharged from the hospital the next day. We expect that the entire study will take about eight months.

To determine whether the virus carrying CFTR is safe and can correct the cystic fibrosis respiratory abnormalities, we will perform several studies on days 1, 3, 7, 14 and 21 after the treatment. We will take swabs from both nostrils and your throat. The nasal and throat swabs are performed by gently rubbing a cotton-tipped applicator in the inside of your nose and in your throat. These procedures are commonly performed to diagnose "strep throat" and are not harmful or painful.

Because this is an experimental virus, there is a possibility that inflammation of the lining of the sinuses or nose may occur. It is possible that redness and pain could occur or that you could develop symptoms similar to those experienced when you have a cold (that is a stuffy or runny nose) or a sinus infection (pain in the cheek, a headache, or a fever). To learn whether the treatments were effective or to learn if any harm was done, we will

biopsy the lining of the sinuses. This will be done after the completion of all the treatments or earlier if we find any evidence of injury from the virus. Prior to the biopsy, an anesthetic to numb the feeling in the area will be used. A small area beneath your upper lip will be used to enter the sinus. This procedure will be done by an Ear, Nose and Throat doctor. This will be done after the completion of all the treatments or earlier if we find any evidence of injury from the virus.

To learn whether the experimental virus can correct cystic fibrosis respiratory abnormalities and can treat the abnormalities in the sinuses that are almost universal in CF, we will obtain computerized tomographic X-rays of your sinuses. We will also measure the voltage across the lining of the nose and sinuses. This involves the insertion of a needle into the skin on your arm, and touching the inner surface of the nose or sinus with a small rubber tube. The rubber catheter and the needle will be connected to a voltmeter. A voltmeter is a recording device and does not deliver any electricity to your nose. Two different drugs at very low doses will be perfused onto the lining of the sinus for approximately 30 minutes. These drugs are terbutaline (which is used to treat asthma), and amiloride (which is used to treat heart and kidney problems). These drugs are approved for human use, and at the low concentration used, do not have major side-effects. To learn whether the treatment was of benefit, we will also study fluid samples taken from the sinuses.

After application of the virus you will be required to be in respiratory isolation for one day. We wish to keep you in isolation so that no one else will be exposed to the virus. This means that you will be confined to a hospital room and visitors will be required to wear protective masks, gloves, and gowns.

About 2 tablespoons of blood will be drawn on the day of admission and discharge and on days 1, 3, 7, 14, and 21. Additional blood samples will be required with the additional six treatments. Pulmonary function tests will be repeated at the end of the study or if there is a change in your disease.

2. <u>FORESEEABLE RISKS AND DISCOMFORTS</u>:

Risks associated with gene <u>transfer</u> using the altered virus are largely unknown. It is possible that the virus could cause an inflammation locally at the site of application in the sinus or it could damage the tissue in the sinus. These problems might produce local pain or bleeding. There is also the very unlikely possibility of scarring in the nose or sinus. It is possible that the virus might reproduce under certain circumstances; this unlikely possibility might lead to spread of the virus and symptoms of a "cold". This may include nasal congestion, runny nose, sneezing, cough or fever. It is possible that a pneumonia could occur, but we think this is very unlikely because we will only apply the altered virus to the lining of the sinus. Other symptoms caused by naturally occurring adenoviruses include sore throat and red, sore eyes. Adenovirus that occurs naturally has been known to cause diarrhea and pneumonia, though these usually are caused by different types of adenovirus than that which was prepared for this study. If a lung infection did somehow occur, then it might cause a worsening of your symptoms of cystic fibrosis. Rare problems caused by adenovirus include an inflammation of the bladder. An extremely unlikely possibility is the formation of a new type of virus through interaction with other viruses. It is possible that participation in the study could cause your immune system to form a response against the altered virus. This could affect your response to subsequent treatments with the altered virus. It is also possible that you may develop an allergic reaction to the virus. The virus you will be exposed to rarely introduces new gene into chromosomes. This makes it exceedingly unlikely that the

altered virus could cause cancer. It also means that correction of the genetic defect in the lining of your sinuses will be temporary.

The method for insertion of the tubes into your nose for washing the sinuses and for application of the virus may be uncomfortable. You will be required to sit still for about one hour without moving your head. The biopsy of the sinus mucosa may be painful and a small amount of bleeding will be expected (about 2 tablespoons). After the biopsy, the healing process takes about 2 weeks and some pain, itching, swelling, and minor bleeding are expected. Pain killers will be available as needed. There is a small risk of infection at the site of the biopsy.

The procedure involved in measuring the voltage on the inner surface of the nose and sinus does not cause pain and is unlikely to produce discomfort other than some itching. Two drugs will be applied to the lining of the nose; each is approved by the Food and Drug Administration for human use. The two drugs used are: terbutaline, which can temporarily increase the heart rate and amiloride, which is a "fluid pill" medication when taken by mouth. At the very low doses that will be applied to the lining of the nose, these medications should not cause problems.

The drawing of blood from a vein or from an artery for blood tests could cause a bruise at the site, minor bleeding, and momentary pain.

We anticipate that you will have up to a maximum of eight computerized tomographic X-rays of the sinuses during the course of the studies. Although there are no known adverse effects of this amount of radiation, the long term effects of such radiation are not known with certainty. The risk associated with the radiation that you will receive from this study is approximately three fifths the annual allowable exposure for a radiation worker.

The altered adenovirus, could be dangerous to an embryo or fetus if you are pregnant; the risks are currently unknown. You will not be allowed to participate if you are pregnant. If you are a woman, we will do a pregnancy test. You will be asked to adopt some form of contraceptive precaution, such as oral contraceptives or an intrauterine device, during this experiment.

During the time that you are participating in this study you will remain susceptible to the problems that are normally associated with cystic fibrosis, such as infections of the lungs. If during the course of this study, you should develop a complication of your cystic fibrosis, we would initiate the appropriate treatment; for example, if you should develop an pneumonia, we would begin treatment with antibiotics. However, in that case, we would ask that you or your insurance company arrange for payment for the therapy.

We do not expect that you will experience any major harmful effects of this study. However, because it could be of importance for future patients, an autopsy would be of value in determining the effects of the treatment. Therefore, in the unlikely event of death from any cause (for example, a traffic accident) during the course of the study, we will request that permission for an autopsy be granted.

3. POTENTIAL BENEFITS TO YOU OR TO OTHERS WHICH MAY BE EXPECTED FROM THE RESEARCH:

It is unlikely that participation in this study will directly benefit you. The correction of the genetic defect of cystic fibrosis will likely be limited to only the actual area where the virus was applied to the sinus. The primary benefit will be a better understanding of the

possibilities for gene therapy with the altered virus in cystic fibrosis. In particular, information regarding the amount of virus needed and the effects of the virus on the cells of the airway will be critical for further studies which might involve giving the virus to the bronchial airways of the lung to treat the disease.

4. ALTERNATIVES THAT ARE AVAILABLE TO YOU:

You may elect not to participate in this study and this decision will not affect your care at the University of Iowa. Treatments of cystic fibrosis include: chest physiotherapy, antibiotics, nutritional and dietary measures, and experimental agents which might reduce the thickness of the mucus. These treatments will also be available to you during and after the study. Since this study will not directly treat your lungs, a decision not to participate will not affect your health.

5. CONFIDENTIALITY:

A record of your participation in this research will be maintained, but this record will be kept confidential. You will be assigned a study number and information will be kept under the number rather than your name. All requests for information will be directed to the University of Iowa Health Science Relations Office to minimize the risk that your identity will be disclosed to the public. Gene therapy is very interesting to the general public. Although every effort will be made to maintain your privacy, it is possible that the news media or other individuals might learn your identify and spread this information, even if you do not want this to happen.

6. DISCLOSURE OF INFORMATION TO REGULATORY AGENCIES:

The U.S. Food and Drug Administration (FDA), National Institutes of Health, and Genzyme Corporation may inspect and copy your medical records relating to this study, and the results of the study will be reported to the sponsor, the FDA and perhaps to other regulatory agencies. This information will be treated confidentially and, in the event of any publication regarding this study, your identity will not be disclosed.

7. RIGHT TO ASK QUESTIONS:

Questions about this research will be answered by either Dr. Michael J. Welsh, M.D., University of Iowa Medical Center, telephone (319) 335-7619 or Dr. Joseph Zabner, MD., University of Iowa Medical Center, telephone (319) 335-7574.

8. RIGHT TO WITHDRAW:

Your participation in this study is voluntary. No penalty or loss of benefits to which you are entitled will occur if you decide not to participate. After enrollment in the study, you may discontinue participation at any time before application of the virus without penalty. Because you will receive a virus for which safety data in humans is not yet available, the risks are not yet fully defined. Therefore, if after having received the virus, you wish to withdraw from the study, you may do so but we request that you continue to return to the clinic for evaluation. If you feel that you are not able to make this commitment, we recommend that you not enter the study.

In addition, Dr. Welsh may terminate your participation in this study at any time he deems appropriate, after he has explained the reasons for doing so and has helped arrange for your continued care by your own physician if that is appropriate. Reasons that he might discontinue your participation could, for example, include a significant worsening of your clinical status unrelated to the experimental procedure.

9. COMPENSATION:

There will be no charge to you for participating in this study nor will you be charged for any tests that we perform. You will be compensated for the time and inconvenience involved in participating in the research in the amount of $75 for each day that you spend in the hospital. In addition, we will reimburse you for your travel expenses to and from the University Hospitals for each day that you come to the clinic for an outpatient visit. The rate will be $0.25 per mile and your parking expenses.

10. IOWA FAIR INFORMATION PRACTICES ACT

The Iowa Fair Information Practices Act (Section 22.11, Code of Iowa) requires that persons supplying information be notified of the use that will be made of the information, which persons outside the University might routinely be provided this information, which parts of the information requested are required and which are optional, and the consequences of failing to provide the information required.

I have discussed the above points, including the information required by the Iowa Fair Information Practices Act, with the subject or the legally authorized representative, using a translator when necessary. It is my opinion that the subject understands the risks, benefits, and obligations involved in participation in this project.

_____	_____	_____	_____
Investigator	Date	Auditor-Witness	Date

FF Principal Investigator/Program Director *(Last, first, middle):* M.J. Welsh and A.E. Smith

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Welsh, Michael J.	Professor

EDUCATION *(Begin with baccalaureate of other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Loras College, Dubuque, Iowa		1967-69	Liberal Arts
University of Iowa, Iowa City, Iowa	B.S.	1970	Gen. Science
University of Iowa College of Medicine	M.D.	1974	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Year	Position	Institution
7/74 - 6/77	Intern and Resident	Internal Medicine, University of Iowa, Iowa City, IA
7/77 - 679	Research Fellowship	Cardiovascular Research Institute, University of California at San Francisco, San Francisco, CA
7/79 - 12/80	Research Fellow	Department of Physiology, Univ. of Texas Medical School at Houston, TX
1/81 - 6/84	Assistant Professor	Pulmonary Division, Department of Internal
7/84 - 6/87	Associate Professor	Medicine, University of Iowa College of Medicine
7/87 - present	Professor	Iowa City, IA
1989 -	Professor	Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA
1989 -	Investigator	Howard Hughes Medical Institute

Year	Honor
1977	Cecile Lehman Mayer Research Award, American College of Chest Physicians
1977	National Pulmonary Faculty Training Award
1983	Established Investigator of the American Heart Association
1983	American Society for Clinical Investigation
1989	American Association of Physicians
1992	Doris F. Tulcin Cystic Fibrosis Research Award

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RECOMBINANT DNA RESEARCH

PUBLICATION HISTORY

Volume	Period	Publication Date	NIH Publication Number
1	February 1975 - June 1976	August 1976	76-1138
2	June 1976 - November 1977	March 1978	78-1139
3	November 1977 - September 1978	September 1978	78-1843
3A	November 1977 - September 1978	September 1978	78-1844
4	August 1978 - December 1978	December 1978	79-1875
4A	August 1978 - December 1978	December 1978	79-1876
5	January 1980 - January 1980	March 1980	80-2130
6	January 1980 - December 1980	April 1981	81-2386
7	November 1980 - August 1982	December 1982	83-2604
8	September 1982 - September 1984	May 1986	86-2863
9	September 1984 - March 1985	May 1986	86-2864
10	March 1985 - December 1985	May 1986	86-2865
11	January 1986 - April 1987	December 1990	91-3203
12	April 1987	December 1990	91-3204
13	May 1987 - December 1988	December 1990	91-3205
14	January 1989 - July 1991	January 1994	94-3708
15	August 1991 - June 1992	January 1994	94-3709
16	July 1992 - December 1992	January 1994	94-3710
17	December 1992 - June 1993	January 1995	95-3898
18	June 1993 - December 1993	January 1995	95-3904

NIH Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant Molecules	October 1977

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Publication No. 95-3904
January 1995